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THE FRANK LEAD EXERCISE ELECTROCARDIOGRAM

A QUANTITATIVE STUDY BASED ON AVERAGING TECHNIC AND DIGITAL COMPUTER ANALYSIS

By

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PURPOSE OF THE STUDY

The rapid development during the last decade of electronic technics for data acquisition and processing has provided the physician with new and powerful tools for the development of more accurate, quantitative and objective methods of analysis and interpretation of clinical data. Electrocardiography is one of the areas where the potential gains ppear particularly large. In present practice vast amounts of data are analyzed by time consuming methods subjected to a sizeable inter and intraobserver variability

Important progress has been made toward a general system of computer analysis and interpretation covering the entire range of clinical electrocardiography but much work remains to be done before an optimal, practical solution is found. The introduction of computers has made available a wide new range of analysis methods capable of providing diagnostic information impossible to extract by conventional methods.

The electrocardiographic exercise test is an important method in the clinical diagnosis of coronary heart disease. The test has also been used as method of uncovering latent coronary heart disease in population studies and insurance medicine. In the latter applications particularly is there growing demand for objective, quantitative methods of analysis.

The present study is an attempt t explore new methods to record, process, and analyze electrocardiographic data from exercise tests. It is primarily pilot-study including number of small experimental groups of patients with coronary disease and controls studied under various conditions to provide material for a study of the relationships between quantitative electrocardiographic and physiological data. It was thus considered desirable to obtain electrocardiographic data during exercise. Averaging technics seemed to offer a means of greatly improving the technical quality of the records.

A major part of the experimental work has been devoted to a study of the electrocardiographic response to exercise in normal subjects, i.e. young and middle-aged men without clinical evidence of cardiovascular disease. A method designed to detect latent disease can only be validated by follow-up studies. The normal groups included in this study are selected and too small to permit any definition of criteria of normal response to exercise. However surprisingly few truly quantitative studies re available on the electrocardiogram during and after exercise in normal subjects It was considered possible that a quantitive study also of a small material might yield some clinically useful information.

Theoretically highly interesting methods of pattern-classification for the analysis of complex data have recently been developed One of these methods Rosenblatt's error correcting training procedure, seemed to offer important advantages over other analysis methods. Its potential usefulness in a diagnostic application has been explored in a study including selected group of patients with angina pectoris.

Section I

INTRODUCTION

Burch et al. (1964) have expressed the opinion that in the future the most usual evolutionary course of a new analytical process or data reduction technic will be that of approximating the technic on a general purpose analog computer followed by more discrete testing on a general-purpose digital computer. After various transforms have been tested and selected the process will be crystallized in special-purpose device

No system for general computer ECG diagnosis has yet been developed along these lines. Some investigators have employed both digital and analog methods to explore new methods, g. Rautaharju (1963) but most groups have been working exclusively with digital or analog methods. Analog methods have been used to transform and implement original data and have generally been supplemented with other analysis methods (Abildskov 1958, Moore 1962, Lamb 1963 Rijlant 1962) Complete data processing systems for general clinical ECG diagnosis have been based on magnetic tape recording, analog-to-digital (AD) conversion, and digital computer analyses (Pipberger and Stallman 1964, Caceres 1964) Pipberger has repeatedly (g. 1960 1964) pointed out the d antages of using general-purpose digital computer during a development stage Once original data have been converted into numerical form and stored in medium acceptable to the digital computer variety of analysis methods can easily be investigated.

The present study has been linked to larger project employing a digital computer and aimed at the development of a complete hospital information system (Hall et al. 1965) It seemed logical to attempt to build an ECG system based on digital computer analysis and to adapt the recording system and the pre-analysis data processing procedure to the requirements of the available computer (SAAB D21) The basic components of the system prior to the digital computer would thus be a magnetic tape recorder and an AD-converter with paper punch tape output.

Recording of original analog data on magnetic tape contributes greatly to the flexibility of the system. On-line AD-conversion is often impractical under experimental conditions at the present stage of technical development, and to avoid the inaccurate and extremely time-consuming method of manual AD-conversion of conventional paper tracings provision must be made for intermediate storage of the analog data in original form.

At the time this study was initiated, no AD-converter with capacity to handle ECG recordings was available locally and there were then no plans of building any large scale unit for processing of biomedical analog data. A small computer of average transients (Mnemotron CAT) with digital output offered within reasonable economical limits an attractive alternative to having the records processed at some distant computer center In addition, it provided means of

CHAPTER I

LEAD SYSTEM

In comparison with the standard 12 lead ECG an orthogonal lead system seemed to offer important advantages and few disadvantages as basis for quantitative ECG analysis. Advantages include primary data reduction by a factor of 4 with probably no loss of diagnostically important information, reduction of interindividual variability among normal subjects, and provision of an empirical basis for vector treatment of amplitude data. The main disadvantages are the limited applicability of data from the literature on conventional ECGs, and technical difficulties. The electrode placement of most orthogonal systems makes them less suited for ECG recording during exercise and in severely ill patients. The introduction of a resistance network necessitates careful skin preparation.

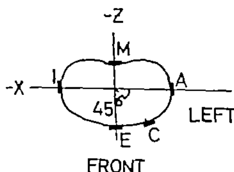


Fig. 1 Frank lead system. Electrode positions. The chest electrodes are placed at the level of the 5th costal interspace at the sternum (sitting position). A head (H) and left leg (F) electrode complete the system.

Frank's system (Frank 1956) has gained the widest acceptance of all suggested orthogonal systems as representing an optimum between partially conflicting requirements of electrical performance and ease in application. The SVEC III system, designed by Schmitt and Simonson (1955) probably has better performance characteristics but requires 14 electrodes. Several other orthogonal lead systems have been proposed. A number of studies have been carried out with the system described by McFee and Parungao (1961). The same theoretical considerations apply to all orthogonal systems.

Frank's Orthogonal Lead System

From the hypothesis that the electrical activity of the heart can be accurately represented by an equivalent dipole it follows that four are the theoretical minimum number of electrodes in any vectorcardiographic system. However a four-lead electrode system is extremely sensitive to variations in heart position and/or electrode placement. Frank's system employs seven electrodes. Five of them are placed at the level of the fifth interspace at the sternum (in the sitting and standing position, at the fourth in supine position) (E) at the front and (M) at the back midline, (I) at the right, and (A) at the left midaxillary line, and (C) at an angle of 45 degrees between (A) and (E). See Fig. 1. The remaining two electrodes are placed on the left leg, (F) and on the back of the neck, (H). In addition, there is

improving greatly the technical quality of the ECG recordings by reducing the amount of random noise.

The system for data acquisition, intermediate data processing, and digital computer analysis described in Section I of this report is primarily an experimental unit and has no capacity of handling large amounts of ECG data at its present state of development.

Chapters I and II deal with the recording system. Chapters III and IV are devoted to pre-analysis data processing methods, including AD-conversion and averaging, computer programs for storage and normalization of ECG data and for selection of variables to be entered in analysis programs. Chapter V deals with analysis methods. Conventional technics are briefly outlined, and a multidimensional pattern-classifying system is described in some detail.

Material

The results reported in this section are based on the recording of more than 1 000 ECGs and the processing of 980 ECGs from 101 exercise experiments in 54 normal subjects and patients with angina pectoris. The material includes 66 ECGs recorded during maximal work.

lar comparison based on 8 digitized records from 4 normal subjects and 4 cardiac patients. The results were identical.

Thus, the modification of the Y lead did not alter appreciably the electrical performance of the lead. Scalar ECG records recorded during exercise with the modified system were comparable to CH leads at work load levels not exceeding 900 kpm/min. At higher intensities the modified Y lead became increasingly noisy.

ST depression in orthogonal and bipolar chest leads. The quantitative relationship between ST depression in scalar orthogonal and conventional leads was studied in a small sample of 15 records during and after exercise in 5 patients with angina pectoris. Horizontal ST depression was present in all leads. The mean amplitude was 0.22 ± 0.03 mV in lead CH_4, 0.13 ± 0.02 mV in lead CH_6, and 0.10 ± 0.01 mV in lead X. The difference between X and CH_4 was significant, but the difference between X and CH_6 was not. The mean amplitude in lead Y was only 0.06 ± 0.01 mV.

The differences between the leads demonstrated consistent trend. The ST amplitude was in each case largest in lead CH_4 and decreased through leads CH_6, X, and Y.

Comments

Biophysical aspects. Biophysical aspects of electrocardiographic leads, the lead vector or transfer impedance concept, and design of lead systems have been reviewed by Schmitt (195).

Okada (1963), Helm and Chou (1964), Geselowitz (1964) and Flowers et al. (1965) have recently critically discussed the theoretical basis for vector electrocardiography.

The theoretical validity of any orthogonal or vector lead systems rests on the assumption that it is possible to represent myocardial electrical activity by a dipole equivalent generator. Early cancellation experiments (Schmitt et al. 1953, Frank 1955) appeared to verify the single dipole concept, but a number of studies have later questioned its validity. Among the technics employed are body surface potential mapping and factor analysis. Factor analysis suggested that all non-redundant information in the QRS complex could be expressed in the form of seven or eight completely different waveforms. Three or fewer principal factors are compatible with a dipole generator but do not prove its existence. The relative information content of the first three factors have been assessed differently in different studies, but it is generally agreed that they contain much more information than factors four to eight (Flowers et al.).

The conclusion is that there is no valid *theoretical* basis for the use of vectorcardiography or orthogonal lead systems. On the other hand, there is no conflict between these theoretical limitations and the clinical usefulness of empirical vectorcardiography (Okada). From clinical point of view the question of the validity of the dipole concept could be restated into a question of whether or not the orthogonal leads contain all clinically useful information present in the standard 12 lead electrocardiogram. This problem has been studied by Pipberger et al. (1961) and by Abildskov et al. (1963). Comparisons have shown a *relatively* close agreement between various orthogonal systems (Pipberger 1959).

Whether or not precordial leads primarily record dipole or "local" potentials represents another aspect of the same problem. Pipberger's and Abildskov's results imply that non-dipole precordial lead potentials are of

ground electrode Electrode positions and interconnections were based on experiments on three-dimensional homogeneous torso models The X lead is derived from electrodes (A) (C) and (I) The midaxillary electrodes (A) and (I) are relatively more important than (C) that has been introduced mainly to correct a backward slant of the lead vector or effective axis The Z lead is derived from all five electrodes at the transverse level and the Y lead from (H) (M) and (F) The role of the (M) electrode is to correct for a forward slant of the lead vector in relation to the vertical body axis. The contribution of each electrode is determined by a resistance network. The relation between the electrodes can be expressed in the following set of equations

$$V_X = 0\,610\ V_A + 0\,171\ V_C - 0\,781\ V_I$$
$$V_Y = 0\,655\ V_F + 0\,345\ V_M - 1\,000\ V_H$$
$$V_Z = 0\,133\ V_A + 0\,736\ V_M - 0.264\ V_I - 0\,374\ V_E - 0\,231\ V_C$$

A coefficient of 1 000 corresponds to a unit resistance, R. Leads X and Z are normalized to the same relative strength as lead Y by shunt resistors of 7 15 and 1 3 R. Frank suggested a unit resistance of 50 000 ohms, which he considered sufficiently high to present any electrical errors due to high skin resistance.

Application

A Frank resistance network was built with a unit resistance of 100 000 ohms to minimize the effect of a high skin resistance It was adjusted to conform to specifications ± 1 0 per cent The system was contained in a shielded box. A switch arrangement provided connections from the output of the resistance network to the amplifier system and to a standard ECG apparatus for recording of scalar orthogonal leads Another switch system on the input side of the resistance network combined potentials from electrodes (E) (C) (A) and (H) to yield leads approximating CH_2 CH_4 and CH_6 for conventional paper recordings A standard cell of 1 35 V proper resistances and a push button switch were used to generate a 1 00 mV signal for calibration. The polarity of Frank s lead Z was reversed to positive for anterior components. The left end of lead X and the inferior end of lead Y kept their assigned positive polarity

Modification of the Y lead The placement of the (F) electrode on the left leg and of the (H) electrode on the back of the neck is not compatible with recording during exercise. A forehead electrode has been used routinely to record bipolar chest leads during exercise (Holmgren and Strandell 1960) It thus seemed natural to move the (H) electrode from the back of the neck to the forehead. Preliminary experiments on different caudal positions for electrode (F) to substitute for the left leg indicated that the middle part of the sacrum provided an electrode site relatively free from motion artifacts.

The effect of these modifications was studied in scalar records from a sample of seven patients displaying definite QRS and ST T abnormalities. Maximal P Q R, S, ST J and T amplitudes were measured to the nearest 0 5 mm. Two complexes were measured in each ECG No significant differences were found. There was no consistent trend, and differences between mean amplitudes were as large in lead X (unaffected by the modification) as in lead Y and Z. No difference exceeded 0 5 mm or 0 05 mV

This study was supplemented with a simu

The large interindividual variability among normal subjects with regard to standard lead ECG amplitudes is apparent in many quantitative studies, e.g. Simonson (1961).

Abildskov and Pence (1956) have demonstrated that the wide range of findings in normal subjects encountered in clinical electrocardiography becomes considerably smaller when corrected leads are used. Pipberger and Lilienfield (1958) and Draper et al. (1964) have presented data to support this finding.

The discussion has been limited to concern mainly the basic information content of orthogonal versus standard leads. From this point of view there is evidence to suggest that orthogonal leads are preferable to standard leads as a basis for an objective, quantitative analysis system.

little diagnostic importance but results from studies employing factor analysis (Flowers et al.) seem contradictory

In this connection reference should be made to a theoretically highly interesting method to eliminate possible distortions due to proximity effects. Rijlant (1962) has designed an orthogonal lead system that uses 72 electrodes equally spaced over the entire torso and a complicated resistance network transforming the potentials in hyperbolic space and generating mathematically correct dipole vector components.

Relation to conventional ECG leads Pipberger et al. (1961) studied 267 randomly selected patients and compared the standard ECG with the orthogonal ECG recorded with the SVEC III system. In 242 patients the features which led to diagnosis in the standard ECG could be completely recovered in scalar tracings of the three orthogonal leads. A major part of the abnormalities not evident in the orthogonal leads were small R waves with little or no R progession in leads V_1 to V_4 and RSR patterns in lead V_1 There was also one case of isolated T wave negativity in leads V_3 and V_4 Eight of the 19 cases with no apparent abnormalities in the orthogonal leads fell outside the range of normality at a numerical analysis of amplitudes Eleven records or 4.2 per cent, remained where clinical information in the standard 12 lead ECG could not be recovered from the three orthogonal leads

The next step in the analysis consisted of a comparison of the standard leads with resolved orthogonal leads. The resolver consisted of a series of sine-cosine potentiometers that allowed mixing of two orthogonal leads in proportions corresponding to the estimated direction of the lead vector of each conventional lead In 18 out of 19 cases in which clinically significant details could not be recovered from 3 basic orthogonal leads, the resolved leads revealed this information. Thus, a complete correlation was found in 99 6 per cent of the total material. The resolved leads were abnormal also in the last remaining case. All ST segment shifts in the standard leads were present in the resolved leads.

Abildskov et al. (1963) performed a similar study on 35 patients and also calculated the per cent contribution of X, Y and Z components to each precordial lead. Orthogonal leads were recorded with the system designed by McFee and Parungao Only one of 240 precordial leads was not matched with a resolved orthogonal lead having the same features judged to be clinically significant.

Blackburn and Rautaharju (unpublished data) studied a large number of individual leads with regard to the effectiveness with which they displayed ST abnormalities Up to 20 leads were recorded in patients with definite ST abnormalities in the resting 12 lead ECG Most of the patients had clinical coronary heart disease. Their data agree with the results of the comparison between bipolar chest leads and orthogonal leads in this study i.e. that the Frank X lead is relatively less effective in displaying ST depressions than e.g leads CH_4 and CH_6 when absolute amplitudes only are considered. Blackburn s and Rautaharju s data also reveal a correlation between QRS and ST amplitudes largely corresponding to estimations of relative lead strength using known dipoles in saline filled torso models. If the ratio between ST amplitude and lead strength is used as an index of sensitivity rather than absolute ST amplitude lead X proves to be as effective as leads CH_{4-6}.

visual comparison of the ECG signal at the output of the preamplifiers and at the output from the tape recorder.

Units for Monitoring, Calibration, and Patient Identification

A simple switch-box connected the preamplifiers and the tape recorder with dual beam calibrated standard oscilloscope (Du Mont 304A) ECG signals could be monitored at the preamplifiers or tape re corder output Simultaneous monitoring of tape recorder input and output was feasible. A telephone dial was connected to generate square wave pulses of 1.0 V These pulses served the dual purpose of providing calibra tion of the tape recorder and a simple analog 3-digit patient and 2-digit ECG identifica tion.

The calibration of the oscilloscope was tested gainst a dry-cell precision battery

Recording Procedure

The patient was sitting upright on a stool. The level of the fifth interspace at sternum was located, and the position of each elec trode was marked with a small amount of electrode paste. The skin was rubbed briskly with wooden spatula or the edge of the electrode until definite local hyperemia was noted.

Standard German-sil er chest electrodes were used (Elema Schönander) The dia meter was 2.5 cm. A modern-type electrode creme was used throughout the study Data on skin resistance were supplied by the ma nufacturer (Elema Schönander) A resistance below 10,000 ohms is regularly achieved with proper pplication.

The (H) electrode on the forehead, and the ground electrode, usually on the right forearm, were fastened with ordinary rubber straps. The sacrum (F) electrode and the chest electrodes were firmly fixed with surgi cal tape. Rectangular pieces of sponge plastic were placed over each electrode. The chest electrodes were then secured with a rubber strap around the thorax, and the sacrum elec trode by another layer of tape. The procedure required pproximately 15 minutes.

The D C. level of the preamplifier output was adjusted to zero and the calibration of the preamplifiers and the tape recorder was checked. The ECG signal was displayed on the oscilloscope in order to discover any D C. drift, A.C. or muscular interference. Data on subject, procedure tape reel identi fication, and footage counter reading were recorded. Patient and ECG identification were recorded in analog form on the tape, together with calibration signals.

A 45 second tape recording was then made. The tape recorder output signal was monitored. Each channel was also frequently checked on the tape recorder voltage meter to prevent any distortion due to overloading of the FM system.

Comments

There are no accepted international or national minimum requirements for modern biomedical recording equipment. The Rese arch Committee on Electrocardiography Vec torcardiography and Computer Applications of the International Society of Cardiology is presently concerned with definition of testing procedures and formulation of general recommendations (Blackburn, personal com munication) The system described in this chapter was assembled according to the arbitrary criteria that its performance should at least equal that of the locally most com mon high-fidelity direct writing electro-

CHAPTER II

ELECTRONIC RECORDING SYSTEM AND RECORDING PROCEDURE

The recording unit consisted of a Frank lead orthogonal system (Chapter I) preamplifiers devices for monitoring and identification, and a multichannel magnetic tape recorder

Preamplifiers

The input specifications of the FM tape recorder required amplification of the ECG signal by a factor of 1 000 A set of three Tektronix Type 122 Low level Preamplifiers powered by a Type 125 Power Supply was used The standard low frequency cut-off point, 0 2 cps, is not satisfactory for ECG recording. It corresponds to a time constant of approximately 1 second, which conforms to AHA specifications but may cause a false ST depression (Lepeschkin 1963) The preamplifiers were modified in the low frequency range to give a time constant of 3 seconds Recordings were made with a high-frequency cut-off 3-db point at 10 KC. The input impedance was 10 megohms paralleled by 50 micro-microfarads and the output impedance 1 000 ohms from a cathode follower The signal to-noise ratio conformed to specifications (better than 40 db)

Tape Recorder

A Sanborn Ampex Model 2000 1/2 inch multichannel tape recorder was used throughout the study The maximal number of tracks is 7 but only 4 channels were used. All were equipped with a frequency modulation record reproduce system. The system conforms to accepted standards with regard to FM carrier frequencies, head arrangement etc. Two tape speeds were used 15 and 7½ ips Initially recordings were made at 15 ips, but the latter half of the material was recorded at 7½ ips The upper cut-off frequency was 1 250 cps at 7½ ips, and 2 500 cps at 15 ips. Provided high quality magnetic tape was used response characteristics conformed to the specifications with a peak to-peak signal ratio of better than 35 db at 15 ips and better than 36 db at 7½ ips and an A.C. non-linearity of ± 1 0 per cent.

Input and output characteristics input impedance 10 000 ohms single ended, input nominal voltage level ± 1 2 to ± 3 V (± 2.5 V was used) output impedance 100 ohms, single-ended. The system was adjusted to unity gain. The inter channel time displacement error was nominally 200 microseconds at 30 ips and the tape speed deviation nominally < 0 25 per cent. The system had provision for monitoring of the signal input and output during recording and was equipped with a footage counter

A careful visual comparison of a set of 10 orthogonal ECGs recorded with a standard 4 channel ECG apparatus (Elema Schönander Mingograf 42 B) and the same leads initially recorded on magnetic tape and then reproduced and fed into the same ECG machine did not reveal any differences.

The performance of the tape system was also checked routinely at each recording by

AVERAGING AND ANALOG-TO-DIGITAL CONVERSION

The reasons for using a digital computer in the analysis of ECG data have been briefly outlined in the introduction to Section I.

AD-conversion, i.e. the transformation of a continuous variation into a series of discrete numerical values, was linked with an averaging procedure to decrease the amplitude of the noise inevitably present in ECGs recorded during exercise. Computer analysis programs are generally more sensitive to noise than is the human observer.

Averaging technics have been applied to electrocardiography by Rautaharju (1963). A preliminary study on exercise ECGs was reported by Blomqvist et al. (1964). Rautaharju and Blackburn have recently (1965) published a detailed study on the same subject. The technic has been used extensively in neurophysiological research projects, especially to study evoked responses obscured by random noise. A recent bibliography issued by manufacturer of an averaging computer listed over 200 publications on this and related subjects.

Averaging achieves an increase in signal-to-noise ratio by serially adding repetitive signals. The addition cycle must be initiated by synchronizing signal that is constantly related in time to the data signal. The coherent portions of the input signal, e.g. ECG waveforms, reinforce each other with each successive addition. The non-coherent noise is added out of phase and therefore tend to decrease with each addition. It can be proved mathematically that the decrease in noise amplitude is proportional to the square root of the number of additions. Mathemathical aspects of the averaging technic with reference to ECG have been discussed by Rautaharju (1963).

Instrumentation

AD-conversion and averaging were performed in one operation by a small, portable special-purpose computer of average transients (CAT 400 B Mnemotron Division, Technical Measurements Corporation).

The necessary synchronizing signal was generated by a separate trigger unit in a special procedure.

Synchronization

The QRS complex is the only part of the ECG that can provide an unambiguous point of reference for timing. If each addition cycle were to be initiated by trigger pulse generated by the R wave of the preceding ECG cycle, serious distortion of data would result from the beat-to-beat variation in R-R interval. It follows that if the analysis is not to be limited to the ST T segment triggering must be based on anticipation of the ECG signal. Such anticipation can be achieved with tape recorded ECG data by *introducing* an extra reproduce head that *reads the re*corded ECG at a fixed interval before the signal reaches the standard reproduce head. The ECG from the extra reproduce head can then be fed to a trigger unit that will instruct the computer to start sampling from

cardiograph (Elema Schönander Mingograf 42 B)

The weakest links of the present system are probably the introduction of a resistance network before the signal is amplified, the signal-to-noise ratio and the low frequency cut-off point of the preamplifiers.

A time-consuming method had to be used to ensure a low skin resistance. In routine application a system of emitter followers or equivalents probably should be incorporated to eliminate a potential cause of serious distortion of data. A time constant of 3 seconds is well above accepted standards for conventional ECG machines, but it nonetheless introduces some distortion of low frequency components. Recent developments of new types of electrodes (silver-silver chloride electrode, Beckman) may provide means of extending the low frequency range.

The frequency modulation technic brings the low frequency response of the tape recorder down to D C. The bandwidth of the tape system is limited by its high-frequency characteristics. The upper cut-off point is directly proportional to the tape speed. The choice of bandwidth will thus greatly influence the running costs. The high cost of heavy duty instrumentation tape was the reason for employing a tape speed of 7½ ips and a cut-off point of 1 250 cps in the latter half of this study

CHAPTER III

AVERAGING AND ANALOG-TO-DIGITAL CONVERSION

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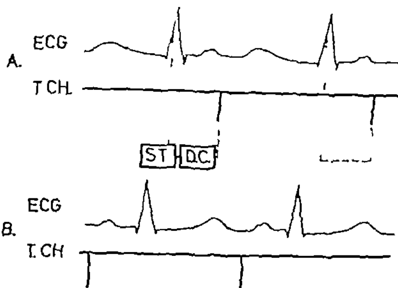
The necessary synchronizing signal was generated by separate trigger unit in a special procedure.

Synchronization

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Fig 2 Procedure providing anticipation

The method generates a trigger pulse preceeding each QRS complex at a fixed distance from the R wave. (A) demonstrates the first step of the procedure. The tape recorded ECG is reproduced *with reversal of the time* base A Schmitt trigger (S.T) is activated by the rapid slope of the R wave. The trigger pulse is passed through a delay circuit (D.C.) and recorded on a separate channel (T.CH) (B) shows the result of (A) Each P wave is preceeded by a trigger pulse that will provid proper start pulse for the averaging computer The trigger-delay circuit unit was designed and manufactured by Mr S Persson, F-a ELEDIA, Stockholm.



the output of the standard reproduce head. *The anticipation interval is a function of the tape speed and the distance between the two heads.* The standard head of some tape recorders *may be rewired to provide anticipation* An extra reproduce head can be fitted to other models.

It proved impossible to apply any of these alternatives to a Sanborn 2000 recorder A special procedure had to be developed.

Procedure providing anticipation The process *is outlined in fig 2* The symmetrical design of the record and reproduce head made it possible *to reproduce the ECGs* with reversal of the time axis

The trigger unit was activated *by the R wave. A delay circuit was inserted after the* trigger The delay was adjusted to approximately 250 milliseconds A trigger pulse could thus be generated and recorded at a fixed distance from each R wave on a channel not occupied by ECG signals When the tape was played back moving in normal direction, each P wave was preceeded by a trigger pulse synchronized with the following R wave. The recorded trigger pulse did not have a sufficiently short rise time to start the CAT computer but could easily activate the trigger unit. The delay circuit was stable Variations in delay time did not exceed ± 0 5 msec. at repeated tests over *100 or more cycles.*

Trigger unit The trigger unit consisted of a Schmitt trigger *It was activated if the signal voltage level exceeds a variable bias* voltage. *It could thus* be adjusted to trigger on the slope *of the R (or S) wave* Filters eliminated to a large extent the influence of base line variations. The accuracy of the *triggering was checked primarily by* direct comparison of the result of averaging with the tape recorded ECG *Any instability of* the triggering causes a smoothing error that will give a rounded-off appearance to an originally *sharply defined* wave form, e g a Q wave Grossly defective triggering caused

an apparent increase in QRS duration, a decrease in peak amplitude and/or complete disappearance of Q wave and small R waves followed by deep S waves.

Averaging and AD-Conversion

Computer function Upon command from the sychronizing signal, the CAT samples the ECG signals at regular intervals and converts each sample into a discrete number of pulses by feeding the input to pulse oscillators sensitive to voltage change. The change in pulse frequency is a linear function of the concurrent changes in ECG voltage. Pulse counts are accumulated in the memory section of the computer where each address or memory unit corresponds to a specific sampling period in relation to the synchronizing signal. The counts generated during each cycle are added to the number previously stored at the same address.

The total number of memory addresses is 400. Each address can accumulate 10^5—1 counts. The CAT can be programmed to sample 1 or 2, or 4 simultaneous input signals over intervals varying from 31.25 milliseconds to 16 seconds. This range corresponds to sampling rates varying between 12 750 and 6.25 per second and input channel. Two alternatives were used in the present study both corresponding to a sampling rate of 200 per lead and second. At heart rates below 120, 2 simultaneous channels were sampled during 1 second, and at heart rate above 120, 4 channels during 0.5 seconds (the fourth channel not being used).

The relation between the number of counts per memory unit and voltage is defined by the following equation.

$$\frac{dn}{de} = \frac{(2.5\,t - 0.036)\,f\ S}{6}$$

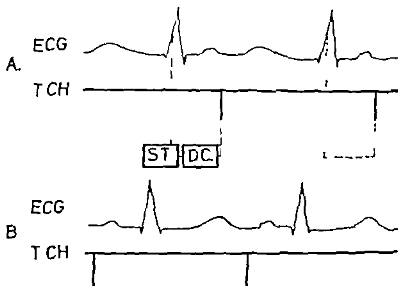
where t, is the analysis time in seconds, f the center frequency of the pulse oscillator of the signal input modulators in KC, and S the number of cycles added.

Application AD-conversion and averaging of a resting ECG in this study typically involved sampling from two simultaneous leads during 20 complete ECG cycles with an analysis time of one second. The modulators had a carrier frequency of 250 KC. A voltage change of 1 mV in the ECG would with preamplifier gain of 1,000 yield 4,173 counts. The nominal nonlinearity of the modulators is less than 0.5 per cent of a full-scale deviation of ± 3 V Modulator preformance was frequently checked by introduction of known D.C. currents. Variations in sensitivity and linearity were below ± 1 per cent.

The recording to be averaged was identified and screened for gross recording errors, e.g. loose electrodes and D.C. level drift. The amplitudes of the calibration pulses in each lead were carefully checked on the oscilloscope. The entire recording system operated with fixed gain and adjustments of the tape recorder output sensitivity were required only occasionally. The number of ECG cycles available for averaging varied with the heart rate — and thus also with the work load level and the noise level — as basic recording time of 45 seconds was used. The median number of cycles used for averaging was 20 at rest 25 during work at the 300—600 kpm/min. level, 45 at 900 kpm/min., and 80 during maximal work. The result of the averaging was displayed on the CAT oscilloscope and examined for residual noise and signs of inadequate triggering. The output of the trigger unit during averaging was routinely recorded on a direct writing ECG apparatus. Gross errors e.g.

Fig 2 Procedure providing anticipation

The method generates a trigger pulse preceeding each QRS complex at a fixed di stance from the R wave (A) *demonstrates the first step of* the procedure. The tape re corded ECG is reproduced with reversal of the time base. A Schmitt trigger (S T) is activated by the rapid slope of the R wave. The trigger pulse is passed through a delay circuit (D C) and recorded on a separate channel (T CH)



(B) shows the result of (A) Each P wave is preceeded by a trigger pulse that will provide a proper start pulse for the a eraging computer The trigger-delay circuit unit was des gned and manufactured by Mr S. Person, F.a ELEDIA, Stockholm.

the output of the standard reproduce head. The anticipation interval is a function of the tape speed and the distance between the two heads The standard head of some tape re corders may be rewired to provide anticipa tion. An extra reproduce head can be fitted to other models.

It proved impossible to apply any of these alternatives to a Sanborn 2000 recorder A special procedure had to be developed.

Procedure providing anticipation The pro- cess is outlined in fig 2 The symmetrical design of the record and reproduce head made it possible to reproduce the ECGs with reversal of the time axis

The trigger unit was activated by the R wave. A delay circuit was inserted after the trigger The delay was adjusted to approxi mately 250 milliseconds A trigger pulse could thus be generated and recorded at a fixed distance from each R wave on a chan nel not occupied by ECG signals. When the tap was played back moving in normal direction, each P wave was preceeded by a trigger pulse synchronized with the following R wave The recorded trigger pulse did not have a sufficiently short rise time to start the CAT computer but could easily activate the trigger unit. The delay circuit was stable Variations in delay time did not exceed ± 0 5 msec. at repeated tests over 100 or more cycles.

Trigger unit The trigger unit consisted of a Schmitt trigger It was activated if the signal voltage level exceeds a variable bias voltage It could thus be adjusted to trigger on the slope of the R (or S) wave Filters eliminated to a large extent the influence of base line variations. The accuracy of the triggering was checked primarily by direct comparison of the result of averaging with the tape recorded ECG Any instability of the triggering causes a smoothing error that will give a rounded-off appearance to an orginally sharply defined wave form, e g a Q wave Grossly defective triggering caused

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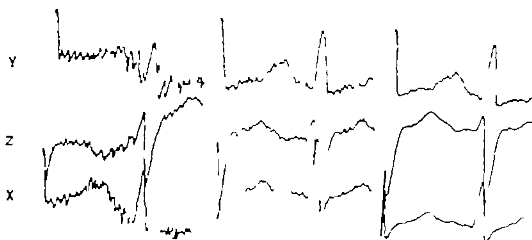


Fig. 3. Signal-to-noise ratio improvement by averaging.
The figure illustrates the decrease in amplitude of random noise by a factor equal to the square root of the number of cycles added.

triggering on ventricular premature beats could easily be recognized and located with the aid of the footage indicator. The corresponding section of the tape could thus be avoided at a re-run of the averaging procedure.

If the result appeared satisfactory, the paper punch unit (Tally) was started. A 3-digit patient and a 2-digit ECG identification were entered manually. The record stored in the CAT memory was then transferred to the paper tape in binary coded decimal form. Each address was represented by a 4-digit identification number and a 5-digit record of the number of counts accumulated.

Excluding the pre-recording of a trigger pulse, processing of one ECG required approximately 5 minutes, often considerably more.

Results

Averaging. The amount of residual noise after averaging and digitizing was estimated in a sample of 100 consecutive records. Measurements were made on large-scale computer print-outs (1 mV = 1.5 cm, 100 msec. = 8 cm). The sample contained 19 ECGs recorded at loads of 900 kpm/min. or higher, 10 were recorded during maximal work. Peak-to-peak noise amplitudes of 0.01 mV or more were present in 9 per cent of all X leads, in 20 per cent of the Y lead and 14 per cent of Z leads. Noise amplitudes exceeding 0.06 mV (corresponding to 0.6 mm in a standard scalar ECG) were present

in 4 per cent of the X leads, 2 per cent of the Y and in 1 per cent in the Z leads. The maximal noise peak in this sample measured 0.08 mV Small amplitude low frequency noise is difficult to assess by simple inspection of ECG recordings, especially after averaging when the part of the record following the end of the T wave may be distorted due to normal variation in R R interval. Cf Fig 4. Obvious D C. level drift was present in 1 lead.

The effect of averaging on very noisy signal is illustrated in Fig. 3 The decrease in noise amplitude by factor equal to the square root of number of cycles averaged is demonstrated An after averaging relatively noise-free ECG can be displayed with high amplification. Details which in standard scalar recordings would have been obscured by random noise are clearly delineated. (Fig. 4)

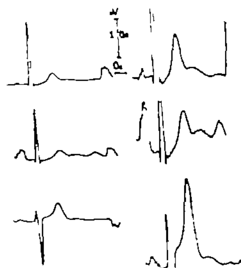


Fig ECG at rest after averag g.

The noise amplitude is sufficiently low to allow display with high amplification. The right part of the figure demonstrates the absence of isoelectric baseline The X lead (top) also shows an initial negative phase of the P wave corresponding to depolarization of the right atrium. The distortion of the second, non-synchronized cycle due to the normal ariation in R R interval is clearly demonstrated by the low-amplitude, rounded P and QRS complex at the end of the left-hand record Sampling time second, peak R amplitude in X 1 8 mV

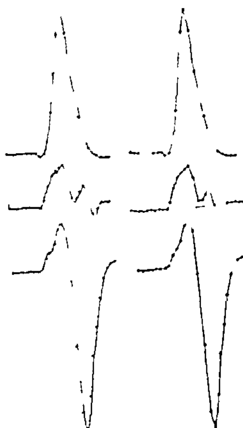


Fig 5 Lo of information due to l w AD conversion rate

Left X, Y and Z leads digitized at ate of 3,200 samples per second and lead. Right: sampling rate 200 per second and lead, linear interpolation The results are also superimposed on the record to the ght Peak R amplitude 1.5 mV in X, analysis time 125 msec.

AD conversion error Fig 5 demonstrates the extent of the loss of information caused by

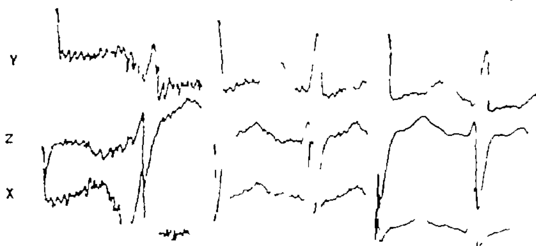


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AD con ersion

Sampling rate The conversion rate must be carefully considered. A low sampling rate may result in significant loss of information, and a high sampling rate generates large amounts of data which poses problems with regard to handling and storage. It is generally desirable to digitize at a rate corresponding to 4 times the frequency of the most high-frequent signal that is to be studied. No standards have yet been developed for AD-convertion of ECGs. Langner and collaborators (1962) have described high-frequency components at 1,000 cps. of possible clinical significance. On the other hand, Berson et al. (1965) found no loss of diagnostic P QRS, and ST T information upon a decrease of the sampling rate from 1 000 to 167 per second and lead. In the present study particular attention has been paid to the low frequent segments of the ECG Dominant frequencies during the ST T interval are mostly below 10 cps. Berson s findings suggest that a rate of 200 cps may have been adequate to cover also the QRS complex.

Procedure AD-conversion was in this study an extremely time-consuming procedure. High-capacity AD-converters linked to digital computers will probably be readily available in the future A solution to long term data administration problems may be offered by development of methods to represent the ECG signal by e.g. a few coefficients of set of orthonormal exponential functions (Young and Huggins 1963) A digital data reduction procedure could thus conveniently be applied to a high-fidelity representation of the signal and present data for subsequent analysis and storage in a concise form.

a relatively low AD-conversion rate. The left part of the figure shows a QRS complex (leads X, Y and Z) recorded with a sampling rate of 3 200 per second and lead and the right part the same complex recorded with the standard sampling rate of this study or 200 samples per second. The main features of the QRS are preserved, but finer details are lost.

Comments

Averaging

Errors introduced by averaging Rautaharju and Blackburn (1965) have defined three types of errors inherent in the method. A *smoothing error* results from inaccurate triggering as has been referred to earlier in this chapter The accuracy of the triggering is difficult to evaluate by direct measurements. Rautaharju and Blackburn found in one experiment that amplitudes sampled from a large number of ECG complexes with respect to a fiducial point on each R wave were approximately normally distributed over intervals with a standard deviation of 2.5 msec. The relative efficiency of the simple checks to limit the smoothing error outlined above was indirectly borne out by the results of the analysis of QRS amplitudes and QRS durations at rest and during work. Changes corresponded to what has been reported in studies based on conventional scalar records.

A *blurring error* results if the signal does not remain constant over the sampling interval. A minute intraventricular conduction disturbance that varies slightly with respiration may be entirely obscured by averaging.

Non-random noise e.g. unidirectional D.C. drift, and ventricular premature beats, may produce artefacts. The checking procedure in the present program was designed to eliminate errors of this type. Coincidence logic pattern triggering circuits can be used to eliminate (or select) extrasystoles. A simple device to exclude premature beats, a variable delay circuit blocking the trigger output for an interval corresponding to the shortest normal R R interval was tested in the present system, but could not be used when the time axis had to reversed during recording of the trigger pulse.

Evaluation of results There is no standardized quantitative method of determining the noise level in electrocardiograms The general practice in electronics is to measure interference or noise amplitude as r.m.s. (root mean square) amplitude and relate it to the signal level. The signal to-noise ratio is commonly expressed in decibels.

Measurement of r.m.s. amplitude is not as appropriate with regard to ECG recording as to audio signals. The relation between peak-to-peak signal and noise amplitudes may give a more reliable estimation of the performance of an ECG recording system. Generally large-amplitude fast, isolated peaks are more difficult to handle in an analysis procedure than continuous noise of lower peak-to-peak but higher r.m.s. amplitude

The methods employed to evaluate residual noise after averaging were crude, but they nevertheless show that it is possible to obtain relatively noise free records by averaging

Fig 3 demonstrates that averaging was an indispensable step in the analysis of some ECGs recorded during maximal work at high external work load levels.

Rautaharju's and Blackburn's report and the experience from this study suggest that averaging greatly facilitates analysis of noisy ECGs but that considerable care must be taken to eliminate inherent errors of the method.

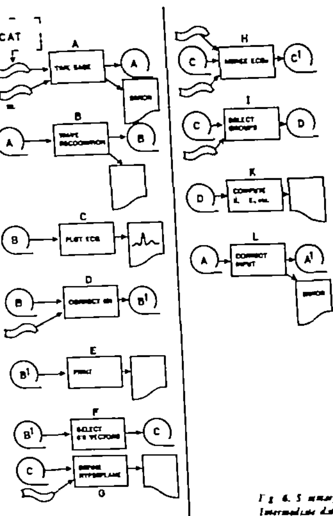


Fig. 6. Summary of digital computer procedures. Intermediate data processing and analysis

Scale factors for amplitude normalization were derived from the relation between the number of counts accumulated in the CAT memory during averaging and input voltage stated in Chapter III, and from data on analysis time and number of cycles averaged entered by a special program.

Selection of reference points

Theoretically the ECG could be analyzed disregarding the physiological meaningfulness of the division into P, QRS, ST and T segments. Such attempts have actually been made (Young and Huggins 1965). The analysis procedure of the present study uses

CHAPTER IV

INTERMEDIATE DATA PROCESSING

The intermediate data processing stage included transfer of primary data from paper punch tape to magnetic tape, time and amplitude normalization, definition of P QRS and ST T segments, and procedures to select variables for analysis

Instrumentation

The same medium-sized digital computer SAAB D21 was used for all digital data processing SAAB D21 is a binary word machine of parallel type with capability of simultaneous input, internal processing and output. It has a cycle time of 4.8 microseconds and an average execution time of 9 6 microseconds. The internal processing is usually overlapped completely by the external units functions Word length is 24 bits and negative numbers are represented by two s complement. The list of instructions consists of 45 machine instructions where each is of single word and single address type It lacks index registers but has two forms of indirect addressing, of which one form partially replaces indexing As a maximum 32 768 words can be addressed The computer used in this study had a core memory capacity of 24 756 words.

External units included

1 punch tape reader 5—8 channels 1 000 characters/second
1 punch tape punch 5—8 channels 150 characters/second
1 typewriter 12 characters/second (output only)
1 printer 1 000 lines/minute
4 tape stations, speed of transfer 288 000 bits/second (50,000 characters/second)

Programs

The design of the complete system for digital processing is outlined in the flow diagram in Fig 6. The large number of separate programs is a result of a gradual development of the system. Mr Torkel Danielsson is the author of all programs. They were written in SAAB D21 language (DAC) but could easily be translated into Algol.

Purely administrative programs will not be discussed.

Time and amplitude normalization

AD conversion of two or more signals simultaneously fed to the CAT computer was carried out by discontinuous sampling from alternate channels When sampling from two channels at a rate of 200 per second and channel, input channel 1 was fed into the AD conversion unit during the first 2 5 msec. interval. Channel 1 was then disconnected, and signal 2 connected and sampled during the following 2 5 msec period. This cycle was repeated throughout the complete analysis period. Sample 1 from channel 1 will thus not be in phase with sample 1 from channel 2 but delayed 2 5 msec This error was corrected by a program using linear interpolation.

QRS Peak amplitude was located in each lead. Maximal X amplitude was selected as reference point if maximal Y or minimum Z amplitude were within ± 50 msec. from peak X. A corresponding combination of Y and Z was used if the conditions were not satisfied by any of the first two alternatives. The beginning of the QRS interval was defined as the first interval before peak QRS that was preceded by a sampling interval with SV < 6.5 μV/sec., and the end point of QRS as the first interval after peak QRS where SV < 6.5 μV/sec.

ST T The critical value of SV for definition of the end of the ST segment and T wa e calculated from mean SV $\overline{SV}$ during the interval (end of QRS — estimated end of T) The estimated end of T n, was computed from the following formula, modified from Simonson (1961)

$$= \frac{2880}{HR} + QRS_{sum} + 29$$

where n defines the desired 5 msec. sampling interval. The formula tends to overestimate QT intervals at low heart rates. HR was therefore set equal to actual heart rate at values of 60 and above, but considered equal to 60 at lower rates.

A minimum duration of 30 msec. was assigned to the ST segment, and the end of the ST segment was defined as the first interval with SV exceeding $\overline{SV}$ if $\overline{SV}$ was also exceeded during at least one of the two following intervals.

The end of the T wave was defined similarly but here the computer started at the interval corresponding to estimated QT + 65 msec. and proceeded stepwise in the direction of the QRS until it found an interval with SV < $\overline{SV}$ where during at least 2 of 3 preceeding interval SV was > $\overline{SV}$ The first interval in this series with SV > $\overline{SV}$ was considered end of T provided the criteria were not fullfilled by any other point within 25 msec. from estimated QT If this was the case, the latter point was selected.

P The search for the start of the P wa e was limited to an interval corresponding to PR intervals between 245 — HR.2 and 85 msec. The mean SV $\overline{SV}'$ during the 150 msec. interval preceding the QRS was computed. The computer started at the beginning of the record and proceeded until it found an interval with SV < $\overline{SV}$ where SV > $\overline{SV}$ during at least 2 of the 3 following intervals. The first interval in this series with SV > $\overline{SV}'$ was considered start of the P wa. If no sample interval within the given limits fulfilled these criteria, the beginning of the P wave defined as the interval whithin the specified extremes where SV reached a minimum.

Base line The base line was defined as the mean voltage of the two samples immediately preceding the QRS complex.

Durations P R, QRS, ST ST T and Q-T durations were measured to the nearest 5 msec.

Results The program was applied to 980 resting and exercise ECGs including several with heart rates > 180 and < 50. The heart rat was 150 or higher in 154 or 16 per cent of all ECGs. The results were checked against large-scale computer print-outs of all ECGs (1 mV corresponding to 100 positions or 125 mm) The computer results were compared to manual measurements supplemented with print-outs of spatial velocity

Apparent discrepancies of less than 20 msec. with regard to beginning of the P wave the end of the ST segment and the end of T wave, and of less than 10 msec.

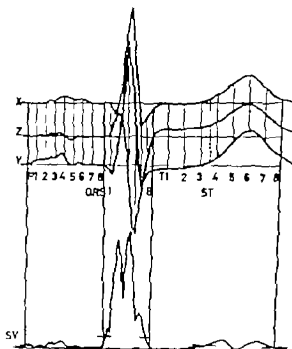


Fig 7 Definition of P R QRS ST and T segments and division into 8/8 P QRS and ST T subsegments

The wave recognition program is based on the spatial velocity (SV) function. The beginning and end of segments is defined by selection of critical values for the SV function.
The figure also shows principles for normalization of the time axis by division of each P QRS, and ST T segment into 8 subsegments of equal duration.

conventional frame of reference e.g definition of a base line for amplitude measurements, and of P QRS and ST T segments

Definition of P QRS and T waves

There is no standard procedure for the exact definition of the beginning and end of the P QRS ST and T segments in clinical scalar records Criteria are commonly based in the magnitude of voltage deviation from the preceding or following part of the record, and on an estimation of the rate of voltage change (Blackburn and Simonson 1957)

The application of computers has provided much more exact methods. The rate of voltage change over a given period of time can easily be calculated from digitized ECGs. Caceres (quoted by Rautaharju 1963) defined the onset of QRS as the sampling interval where the absolute value of the first derivate exceeds 1.875 μV/msec.

Stallman and Pipberger (1961) have based a wave recognition program on spatial velocity (SV) e.g the rate of voltage change calculated from three orthogonal leads

$$\Delta x = x\,(t + \Delta) - x\,(t)$$
$$SV = \frac{1}{\Delta t} \sqrt{\Delta x^2 + \Delta y^2 + \Delta z^2}$$

They stated that spatial velocities exceeding 3 μV/msec. are found only during the P QRS, and T waves, and not during the T P and P R intervals Peak spatial velocity was used as a reference point.

By instructing the computer to scan the record systematically in forward and backward direction for critical spatial velocities they were able to define intervals correctly in 98 7 per cent of the cases in a series of 395 records.

The present study also employed a wave recognition program based on spatial velocity

Wave recognition program

The wave recognition program operated on non-filtered records after time and amplitude normalization. The interval between two consecutive 5 digit amplitude measurements was 5 milliseconds.

Spatial velocity was computed for each sampling period Fig 7 displays a typical spatial velocity function and illustrates the basic principle of the wave recognition program.

Attention paid to the sign of the deriva tive may also prove helpful, i.e. definition based on

$$\Delta SM = \sqrt{x^2(t) + y^2(t) + z^2(t)} - \sqrt{x^2(t+\Delta t) + y^2(t+\Delta t) + z^2(t+\Delta t)}$$

or an approximation of the first derivative of the spatial magnitude (SM) function, rather than SV

The critical value of spatial velocity during QRS, 6.5 μV/msec., is twice as high as that employed by Stallman and Pipberger. A critical SV of 3 μV/msec. resulted in a large number of errors at rapid heart rates. The change in spatial velocity at the onset and end of QRS is generally sudden, and in resting ECGs and ECGs recorded during and after light or moderately heavy exercise 3 and 6.5 μV/msec. gave identical results in most cases. Discrepancies of 5 msec. were occasionally found.

The linear equation for the calculation of estimated Q-T duration was only intended as working device. Extreme prolongation of the Q-T interval would result in erroneous definition of the end of the T wave but is probably rarely encountered in patients subjected to exercise tests. If necessary this point of the program could be modified.

In summary the performance of the wave recognition program was not satisfactory with regard to the definition of the be ginning of the P wave. Errors exceeding 30 msec were present in 62 of 980 ECGs or in 6.3 per cent.

A filtering procedure should probably be applied also to averaged ECGs with comparatively low noise level. A wave recognition program based on the first derivath of the spatial magnitude function rather than on spatial velocity should be developed.

Comments on the selection of reference points

A few general comments should also be made on the essentially conventional frame of reference that has been employed in this study i.e. definition of a base line and division into P QRS, and ST T segments

Base line Caceres (1963) has convincingly demonstrated that the notion of an isoelectric ECG base line at any interval between the end of the U wave and the beginning of the P wave is valid only in ECGs recorded with an amplification sufficiently low to obscure small low frequency voltage changes. Fig. 4 Chapter III supports this view

Definitions of the ECG base line as the level of the P-R or T P segment are also arbitrary from a physical point of view Conventional electrodes and electrode paste result in an ECG signal superimposed on a D.C. level that often is considerably higher than the peak-to-peak amplitude of the ECG signal

The selection of a theoretically satisfactory base line is further complicated by the fact that the ECG during most of the cycle represents mixture of ventricular and atrial potentials in unknown proportions.

The superimposition of atrial activity on QRS-ST T potentials is obvious at high heart rates, but present also at lower rates. In a study on P and T in a material of patients with complete A V block examined at rest and during exercise (Edhag and Blomqvist, unpublished data) a T duration of 0.40 sec. was frequently found. At atrial rates above 120—130 the end of the T wave as a rule coincided with the beginning of the P wave. No studies have been made using D.C. amplifiers and high amplification. The true duration of T is thus not known.

with regard to the beginning and end of the QRS were disregarded.

The weakest point of the system was the definition of the beginning of the P wave. Discrepancies were recorded in 128 records or in 13.3 per cent. Thirty-one of the ECGs in this group were recorded during maximal work. In 66 records the computer results were within ± 30 msec of the true beginning of the P wave which leaves 63 per cent or 62 records containing gross errors. Twenty of these were recorded during maximal work and the heart rate was above 150 in a majority of the remaining records in this group.

There was no record with an incorrect definition of the beginning of QRS and only one with an error in the end of QRS. The end-point of the ST segment was incorrectly defined in 13 ECGs and the end of the T wave in 35 including 16 recorded during maximal work. In the total material 33 records contained two or more apparent errors and 118 simple errors including 92 isolated P wave errors.

Of 116 resting ECGs 16 or 13.5 per cent contained wave recognition errors. Faulty definitions of the start of the P wave occurred in 12 cases and of the end of the ST segment in 4 cases.

Comments on the wave recognition program

The method of evaluating the performance of the wave recognition program can only give approximate results. Print-outs of spatial velocity were used to facilitate the identification of errors possibly due to interference in the records. The resolution limit set by the available 120 positions of the printer may have resulted in some misinterpretation of correct computer results.

The selection of criteria in the wave recognition program may be criticized on several points. The upper limit of the PR interval will exclude cases of AV block I but was considered convenient for the analysis of the present material. It could easily be modified if the program were to be applied to unselected populations. The relation of maximal PP duration to heart rate was derived from data reported by Bengtson (1956). The lower limit of 80 msec will result in an underestimation of the true PP duration in some cases, but it was chosen to satisfy the condition that the sum of PP and QT should be equal to or less than the cycle length. The identification of the end of the T wave was given preference over the beginning of P. No detailed analysis of the P wave should therefore be attempted at heart rates above 160, as the two waves then almost always are superimposed to some degree.

At high heart rates, e.g. during maximal work, in normal subjects lead Y will display a large P wave and a small T wave, whereas lead Z will show a minimal P and a large T wave.

It may thus be possible to achieve a more reliable estimation of PP duration if the condition PP + QT ≤ RR is abandoned.

The delineation of the beginning of the P wave was not satisfactory. Several modifications of the program were tried but no single solution could be found. The computation of mean spatial velocity over an interval corresponding to an average PP duration resulted in some improvement as compared to the use of a fixed interval, but it probably should be supplemented with some smoothing procedure.

Stallmann's and Pipberger's high frequency rates were achieved on filtered records with an upper cut-off point below 60 cps.

Attention paid to the sign of the derivative may also prove helpful, i.e. definition based on

$$\Delta SM = \sqrt{x^2(t) + y^2(t) + z^2(t)} - \sqrt{x^2(t+\Delta t) + y^2(t+\Delta t) + z^2(t+\Delta t)}$$

or an pproximation of the first derivative of the spatial magnitude (SM) function, rather than SV

The critical value of spatial velocity during QRS, 6.5 µV/msec., is twice as high as that employed by Stallman and Pipberger A criti cal SV of 3 µV/msec. resulted in a large number of errors at rapid heart rates. The change in spatial velocity at the onset and end of QRS is generally sudden, and in resting ECGs and ECGs recorded during and after light or moderately heavy exercise 3 and 6.5 µV/msec. gave identical results in most cases Discrepancies of 5 msec. were occasionally found.

The linear equation for the calculation of estimated Q-T duration was only intended as working device. Extreme prolongation of the Q-T interval would result in erroneous definition of the end of the T wave but is probably rarely encountered in patients subjected to exercise tests. If necessary this point of the program could be modified.

In summary the performance of the wav recognition program was not satisfactory with regard to the definition of the be ginning of the P wave. Errors exceeding 30 msec were present in 62 of 980 ECGs, or in 6.3 per cent.

A filtering procedure should probably be pplied also to averaged ECGs with comparatively low noise level. A wave recognition program based on the first derivative of the spatial magnitude function rather than on spatial elocity should be developed.

Comments on the selection of ference po nts

A few general comments should also be made on the essentially conventional frame of reference that has been employed in this study i.e. definition of a base line, and division into P QRS, and ST T segments.

Base li Caceres (1963) has convincingly demonstrated that the notion of an isoelectric ECG base line at any interval between the end of the U wave and the beginning of the P wave is valid only in ECGs recorded with an amplification sufficiently low to obscure small, low frequency voltage changes. F g. 4, Chapter III, supports this iew

Definitions of the ECG base line as the level of the P R or T P segment are also rbitrary from a physical point of view Conventional electrodes and electrode paste result in an ECG signal superimposed on a D C. level that often is considerably higher than the peak-to-peak amplitude of the ECG signal

The selection of a theoretically satisfactory base line is further complicated by the fact that the ECG during most of the cycle re presents mixture of ventricular and atrial potentials in unknown proportions.

The superimposition of atrial activity on QRS-ST T potentials is obvious at high heart rates, but present also at lower rates. In a study on P and T in a material of patients with complete A-V block examined at rest and during exercise (Edhag and Blomqvist, unpublished data) a T duration f 0.40 sec. was frequently found. At atrial rates above 120—130 the end of the T wave as a rule coincided with the beginning of the P w ve. No studies have been made using D C. amplifiers and high amplification. The true duration of T is thus not known.

with regard to the beginning and end of the QRS were disregarded

The weakest point of the system was the definition of the beginning of the P wave. Discrepancies were recorded in 128 records or in 13 3 per cent Thirty-one of the ECGs in this group were recorded during maximal work. In 66 records the computer results were within $\pm$ 30 msec. of the "true" beginning of the P wave which leaves 6.3 per cent or 62 records containing gross errors. Twenty of these were recorded during maximal work, and the heart rate was above 150 in a majority of the remaining records in this group

There was no record with an incorrect definition of the beginning of QRS and only one with an error in the end of QRS. The end point of the ST segment was incorrectly defined in 19 ECGs and the end of the T wave in 38 including 18 recorded during maximal work. In the total material 33 records contained two or more apparent errors, and 118 single errors, including 92 isolated P wave errors.

Of 116 resting ECGs 16, or 13.8 per cent contained wave recognition errors. Faulty definition of the start of the P wave occurred in 12 cases and of the end of the ST segment in 4 cases

Comments on the wave recognition program

The method of evaluating the performance of the wave recognition program can only give approximative results Print-outs of spatial velocity were used to facilitate the identification of errors possibly due to interference in the records The resolution limit set by the available 120 positions of the printer may have resulted in occasional misclassifications of correct computer results.

The selection of criteria in the wave recognition program may be criticized on several points The upper limit of the P R interval will exclude cases of AV block I but was considered convenient for the analysis of the present material It could easily be modified if the program were to be applied to unselected populations. The relation of maximal P R duration to heart rate was derived from data reported by Bengtson (1956) The lower limit of 85 msec. will result in an underestimation of the true P R duration in some cases, but it was chosen to satisfy the condition that the sum of P R and Q-T should be equal to or less than the cycle length. The identification of the end of the T wave was given preference over the beginning of P No detailed analysis of the P wave should therefore be attempted at heart rates above 160 as the two waves then almost always are superimposed to some degree.

At high heart rates e.g during maximal work, in normal subjects lead Y will display a large P wave and a small T wave, whereas lead Z will show a minimal P and a large T wave

It may thus be possible to achieve a more reliable estimation of P R duration if the condition P R + Q-T < R1R is abandoned.

The delineation of the beginning of the P wave was not satisfactory Various modifications of the program were tried, but no simple solution could be found. The computation of mean spatial velocity over an interval corresponding to an average P R duration resulted in some improvement as compared to the use of a fixed critical spatial velocity but probably should be supplemented with some smoothing procedure

Stallman s and Pipberger s high recognition rates were achieved on *filtered records* with an upper cut-off point below 60 cps.

vals defining the beginning and the end of P QRS ST and T

It is difficult to conceive any analysis program with a final or diagnostic stage operating on such large amounts of data. Demands on computer capacity would be extremely high. Some form of data reduction must be carried out before analysis.

The data reduction procedure consisted in this study of normalization of the time base by division of each of the three intervals (beginning of P — beginning of QRS) (QRS) and (end of QRS — end of T) into 8 subsegments of equal duration, and calculation of X, Y and Z lead amplitudes at the end of each subsegment (Fig. 7) The amplitudes were computed after an initial procedure generating by parabolic interpolation 8 amplitude measurements per original 5 msec sample to provide a correct time basis. Amplitudes at the beginning of P and at the end of ST and R R, P-R, QRS, ST ST T and Q-T durations were also included into the final set of measurements which thus consisted of 26 amplitudes from each orthogonal lead, or total of 78 and 6 durations

The points defining the subsegments have been referred to as P 0, P 1 P 8 QRS 1 QRS 8 ST T 1 ST T 8 It should be noted that P 8 coincides with the beginning of QRS and QRS 8 with the ST junction. Amplitudes at these points have in certain applications been looked upon as the cartesian coordinates or vector components of the corresponding instantaneous dipole vector The term 8/8 QRS etc. vectors designates complete set of X, Y and Z coordinates for the segment, and the maximal QRS etc. vector is the vector that has the largest spatial magnitude of those within the set.

Comments

Various methods have been used to select data to form the basis of decision procedures of computer analysis programs They range in remoteness from traditional methods from measurements of P Q R, S, ST and T amplitudes and durations from digitized standard 12 lead ECGs to be entered into an analysis program based on conventional methods of ECG interpretation (Caceres 1963) to representation of the ECG signal by a set of coefficients of orthogonal exponential functions to which linear regression technics are applied (Young and Huggins 1965)

Rautaharju et al. (1961) suggested a representation of data recorded with orthogonal leads based on normalization of the time base within each QRS etc. segment to 1 unit period and division into a number of subsegments of equal duration. This procedure decreased considerably interindividual variation with regard to vector magnitude and direction among normal subjects, as compared to conventional vectorcardiographic methods with sampling at fixed intervals. Sampling at fixed intervals may result in distributions, e.g. at 60 msec. from the beginning of QRS, including instantaneous vectors from the middle part of QRS in subjects with QRS durations approaching 120 msec., and terminal vectors from subject with QRS durations of 80 msec or less. Variations in P R and ST T duration have corresponding effect.

Pipberger and collaborators have made extensive use of the principle of sampling ECG data for analysis after normalization of the time base. Draper et al. (1964) confirmed that time normalization decreases normal ranges Pipberger and Stallman (1964) found that 8/8 instantaneous vectors

Simonson (1961) has discussed errors introduced by the selection of the end of the P R segment as reference level for the ST junction and the end of the T P segment for the ST segment T wave, U wave and P wave as suggested by the American Heart Association, but no alternative is presented

The usage of the end of the P R segment as a common reference level for all amplitudes in this study means that P and T amplitudes have been partly determined by the amplitude of the T wave The obvious superimposition of P and T waves at *high* heart rates made the introduction of *two separate referernce levels* seem unsatisfactory The P R base line level should be considered arbitrary *It* has the practical advantage of being easily defined in a computer program, and the theoretical advantage that in this point ventricular activity approaches zero

In a strict sense, definitions of the beginning and end of the P QRS, and T wave are also arbitrary Detailed studies of the spread of excitation in the myocardium (e.g. Sodi Pallares 1956, Hoffman and Cranefield 1960) have clearly demonstrated that the ECG recorded with remote electrodes represents a display of average potentials from cells in different phases of the activation process

The whole repolarization period has been treated as a unit in this study There are no electrophysiological data to support a division into a ST segment and a T wave The ST segment does not correspond to the plateau of the action potential of a single *myocardial* cell (Hoffman and Cranefield)

Rautaharju (1963) pointed out that ST segment and T wave *durations do not show* the same variation with heart rate Different definitions of the ST segment — T wave junction may modify these relationships

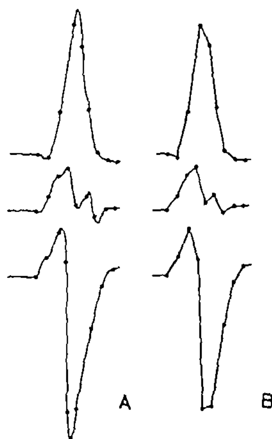


Fig 8 Lo[illegible] f information d[illegible] i [illegible] p [illegible] of the ECG by 8/8 X Y and Z coordinates
(A) demonstrates X, Y and Z leads sampled [illegible] an *AD-conversion rate of 3,200 per lead and second*, (B) 8/8 X, Y and Z coordinates and linear *interpolation Points in* (B) are also superimposed on the ECG in (A) Cf Fig. 5 Chapter III

Selection of Variables for Analysis

The *net* result of the procedures so far described was a set of measurements from each *ECG recording consisting* of a row of 600 (at heart rates below 120 300) five digit amplitude measurements *from* the P R base line six durations, and information on the localization of the *five sampling* inter

CHAPTER V

ANALYSIS METHODS

That the digital computer only too readily lends itself to any desired numerical analysis procedure has probably been an experience common to many clinicians employing computers in attempts to define more accurate methods for evaluation of complex clinical and laboratory data

The main difficulties pertain to the selection of variables to provide a basis for the analysis, and to the definition of an analysis procedure that will transform even large numbers of measurements into concise, clinically useful information. Usually these two problems are considered separately Variables are often selected after pilot studies evaluating various alternatives.

Definition of the analysis procedure commonly includes specification of normal limits and sometimes allocation of weight factors to variables. Thus, the analysis procedure normally operates with pre-determined set of criteria.

Aspects of selection of measurements and selection of analysis procedure are highly interactive

Interesting technics have recently been developed that in certain applications will provide an analysis method and simultaneously attack problems of selection of measurements for analysis. One of these technics in Rosenblatt s error-correcting procedure.

Linear Pattern Classification and Trainable Pattern-Classifying Systems

Nilsson (1965) has reviewed the history and bibliography of Rosenblatt and related methods in a monograph on learning machines. Rosenblatt s method belongs to a group f *trainable pattern-classifying systems*

The Perceptron Rosenblatt successfully combined two concepts proposed during the 1940s and off-on threshold device that was a simplified model of a nerve cell, and the idea that long-term memory in animals depended on changes in the synaptic junctions between neurons. He developed a random network theory on which a brain model was based, the Perceptron. I some experiments the Perceptron was used as a character recognition machine. An important feature of the system was an error-correcting procedure which obviated the need of specifying criteria in advance The program developed criteria during a training period. During this phase the machine was informed of each error in classification. Weight factors assigned to each variable were modified according to fixed principles until the combination of factors and measurements yielded results which classified correctly all members of the training sample, e g. the letters of the alphabet.

The mathematical basis of Rosenblatt s error-correcting training procedure was later formulated by Novikoff The proof has recently been presented in detail by Greenberg and Konheim (1964)

Nilsson s monograph (1965) on which part f this chapter is based, describes basic concepts and mathematical structure of number of trainable systems, parametric and

3

had over a wide range of clinical entities the greatest diagnostic discrimination power of *any set of QRS measurements* included in their study However the difference between 8/8 vectors and a set of 5 initial and 5 terminal instantaneous vectors was small It should be pointed out that the 5 terminal vectors had been measured at 10 msec. intervals from the end of the QRS complex to minimize phase distortion.

Similar comparisons with regard to the ST T segment also suggested superiority of 8/8 vectors (Pipberger et al 1963) Corresponding data on the P wave have not been reported.

Pipberger and Stallman (1964) have presented *evidence that 8/8 vectors provide a* satisfactory basis for an analytical computer program aimed at differential diagnosis *Rautaharju (1963) has also successfully* used representation by 8/8 instantaneous vectors

Data *have* thus been reported supporting the validity of the 8/8 vector concept as a method of data reduction for the QRS and ST T segment Inclusion of the P wave in the analyses was considered desirable for the purpose of this study The 8/8 vector method was applied also to the P wave to obtain uniform data from the whole ECG cycle. 8/8 vectors have generally been presented as X Y and Z vector components, e g scalar X Y and Z leads. The characteristics of this method of display are apparent from a number of illustrations in Section II Fig 8 demonstrates the extent to which finer details of the QRS complex are lost in the data reduction process.

Repeat Variability

The total methodological error was studied by examining the repeat variability in ECGs recorded during exercise in a group of 16 middle aged men without history symptoms or signs suggesting cardiovascular disease (Group OC, Chapter VI) The men were studied twice at a work load of 600 kpm/min with an average interval of 14 days. The total repeat variability will thus include biological variability and the compounded errors of the recording and analysis procedures

Standard error of measurement was calculated for X, Y and Z components of P QRS, *and ST T vectors 3 It amounted to* 0 02 mV for P 3 X and Z components, and to 0 04 mV for the Y component. Mean X, Y and *Z P 3 amplitudes were 0 05 0 29* and —0 02 mV Mean QRS 3 amplitudes were 0.93 0 61 and 0.20 mV in X, Y and Z. Corresponding errors of measurement were 0 19 0 16 and 0.21 mV ST T 3 mean amplitudes were 0 05 0 04, and 0 09 mV and corresponding errors of measurement 0 03 0 02 and 0 03 mV Subsegment vector 3 represented average variability

Thus, the error of measurement for P and ST amplitudes corresponded to 0 2 to 0 4 mm in scalar records and for QRS amplitudes to 1 6 to 2 mm.

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side of the hyperplane. A single linear discriminant function, $g(X)$ is employed for classification of patterns into two categories, e.g. 1 and 2.

The number of dimensions is extended to $d + 1$

$g(X)$ is defined by

$$g(X) = w_1x_1 + w_2x_2 + \quad + w_dx_d + w_{d+1} \quad (1)$$

If $g(X) > 0$, the pattern $X = x_1 + x_2 \quad x_d \; 1$ is classified as belonging to category 1 and if $g(X) < 0$ to category 2. Factors $w_1 \; w_2$ re referred to as weight factors.

The normal euclidean distance from the hyperplane to a point representing an arbitrary pattern X is expressed by

$$\frac{1}{N}(w_1 \;_1 + w_2 \;_2 + \quad w_dx_d + w_{d+}) \quad (2)$$

where

$$N = \sqrt{\sum_{i=1}^{d} w^2}$$

Selection of Discriminant Functions

Discriminant functions can be selected in variety of ways. Sometimes sufficient prior knowledge exists about the patterns to be classified to permit precise calculation. At other times no such information is vailable, or it may be considered desirable not to use any prior knowledge. Discriminant functions may then be defined by training procedure.

Characteristically trainable pattern-classifying system will be able to generate its discriminant functions from the results f various modifications in sample of patterns in which desired classifications are assumed to be known. The patterns in the training set are generally chosen as typical of those which ultimately are going to be classified.

Linear erro -correcting t aining proc dure

The linear error-correcting training procedure (ECTP) basically consists of step-wise adjustment f the weight factors $w_1 \; w_2$ w_{d+1} of Eq. (1) until each member of the training set is correctly classified as belonging to category 1 or 2.

Each set of weight factors defines a hyperplane and the complete ECTP can be described as as sequential examination of a set of hyperplanes (Nilsson). Patterns from the training set are presented one at a time for trial by cycling through the sample. Each passage through the complet training set is called an iteration.

The trial consists of comparing the actual results of $g(X)$ (Eq. 1) with the desired response. If the classification is erroneous, a new set of weight factors i defined by subtracting (if the pattern was on the positive side of the hyperplane) or adding (if the pattern was on the negativ side) the components of the pattern to the corresponding weight factors. This may or may not actually correct the error for the pattern, and it may also undo correction made on a previous pattern. If the classification is correct, no change is made. The process continues until solution is reached, i.e. until a complete iteration does not change the weight factors.

The principle has been illustrated with a two-dimensional space in Fig. 10 and b. A Fig. 10 is a set of patterns in the training set belonging to category 1 for which $g(X)$ should be positive. B belongs to category 2, and a negative response of $g(X)$ is desired. Fig. 10 b demonstrates the steps in the training procedure. The dividing lines 1 2 and 3 correspond to three hyperplanes in multi-dimensional system.

From the logic of the procedure it follow

nonparametric. Rosenblatt's system belongs to the latter group

Basic concepts A pattern, X, may consist of a set of numerical data, e.g. a series of ECG amplitudes or a number of results from laboratory tests $X = x_1\ x_2 \quad\quad x_d$ Individual numbers $x_1\ x_2,$ are called the *components* of the pattern Any device for sorting patterns into *categories* e.g diagnostic entities, is called a *pattern classifier*

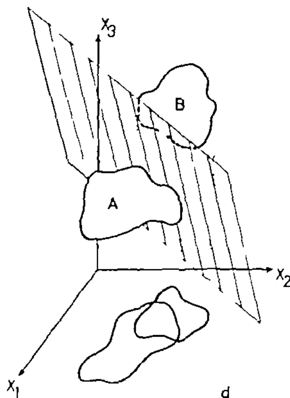


Fig 9 R pre entation f t f three me urements by point a three-d mensional pace

Three measurements, x_1 $_2$ and x_3, characterize each pattern, .g ECG ampl tudes (A) and (B) represent the equiprobabil ty urfaces of sets of patterns, g patients nd controls. A complete separation of the 2 groups cannot be achieved by evaluating only variables x_1 and The optimal discriminant function, d, results in overlapping If third dimension is added, complete separation becomes possible The plane between (A) and (B) represents decision surface and can be regarded as analog to the separating hyperplanes in multidimensional space defined by linear pattern classification procedures.

Some aspects of pattern classification are conveniently discussed in geometric terms. A pattern where $d = 3$ can be represented by a point in a three-dimensional space. The position of the point is defined by three rectangular coordinates $x_1\ x_2$ and x_3 It is impossible to visualize a space with more than three dimensions, but there are no mathematical laws preventing an extension of the number of dimensions The pattern $X = x_1\ x_2 \quad\quad x_d$ can thus be represented as a point a d-dimensional space with coordinates $x_1\ x \quad\quad x_d$

The d-dimensional space can be divided into *decision regions* one for each category of patterns The decision regions are separated by *decision surfaces*

Fig 9 illustrates a three-dimensional space with two sets of patterns represented by equiprobability surfaces A and B Each pattern is characterized by its $x_1\ x_2$, and x_3 coordinates The plane separating A and B represents a decision surface.

Decision surfaces are defined by *discriminant functions* They may be of varying complexity Only linear discriminant functions will be discussed.

Linear classification of patterns In multidimensional space decision surfaces defined by linear functions consist of *hyperplanes* Linear discriminant functions may theoretically be defined to separate patterns into any finite number of categories, but only a linear separation into two categories will be considered

Two sets of patterns are linearly separable *if and only if* a hyperplane exists which has each member of the first set on one side and each member of the second set on the other

been proposed and tested on ECG data by Okajima et al (1963)

In the present study an attempt is made to utilize two aspects of ECTP Patterns have been defined by series of ECG amplitude measurements, and sets of patterns by e g. groups of patients and controls

) Each weight factor measures the importance f the corresponding pattern component in separating sets of patterns, e.g. defines the diagnostic contribution of each ECG amplitude measurement.

b) Theoretically a large number of ECG amplitude measurements could be reduced to single numerical value defining quantitatively the position of each individual ECG in relation to other normal or abnormal records by the distance to the separating hyper plane.

ECTP has been used for intra and inter group comparisons. Intragroup comparisons were carried out mainly to study relationships between work load and ECG response and intergroup comparisons to study age differences and differences between control subjects and patients with angina pectoris

Two sets of pattern components have been employed. The first type included cartesian X, Y and Z coordinates for 25 dipole vectors (beginning of the P wave and 24 1/8 subsegment vectors of the P R QRS, and ST T segment) of each orthogonal ECG thus d = 75

The second type of pattern components was defined by the results of type 1 comparisons It included X Y and Z coordinates of QRS vectors 6 7 and 8 and ST T vectors 1 through 6 The total number of measurements was thus 108.

The results of each comparison were checked by the introduction of a test group

Computing procedure Appropriate identi fication data were entered on paper punch tape to define groups and to assemble relevant amplitudes from the magnetic tape containing the total material of the study In addition to the two groups to be separated, A and B third group was always introduced to test the discriminant function. This principle is illustrated in Fig. 10 d.

The computer was instructed to determine weight factors for = 0. If linear separation proved possible was increased by steps of 10 units each time a solution was found. Practical considerations, mainly cost of computer time, limited the number of iterations. 1,000 was considered reasonable and corresponded to approximately 5 minutes of SAAB D21 time. The program was then interrupted, and the results printed out.

The results included in normalized form the weight factors defining the optimal hyperplane, and the distance to the separating hyperplane for each ECG in the A, B, and test group

Comments

Results are discussed in Section II, but a few comments should be made on inherent advantages and disadvantages with regard to ECG data. The main advantages are.

1 The method is non-parametric.
2. Comparisons can be based on large numbers of ECG amplitude measurements.
3 No criteria have to be defined priori.
4. Sample programming.
5 Quantitative estimation of the relative gnificance of each ariable.
6. Quantitative estimation of the strength of any individual association with two (or more) classes or diagnostic groups.

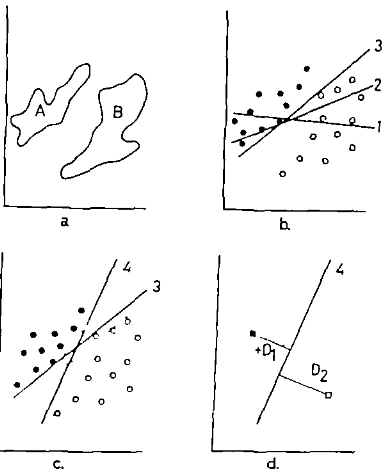


Fig 10 Principles of the error correcting procedu e

The principles are illustrated in a two-dimensional space The process has actually been carried out in a 76- or a 109-d mensional space The l nes n the f gure correspond to hyperplanes in multi-dimensional space.

(a) illustrates two sets of pat terns w th a non-normal distribution, e g ECG amplitudes in patients and controls.

(b) shows three steps in the process by which weight factors defining the separati g function are mod fied to achieve separa tion n step 3 Modif cations are effected by adding t o subtracting from the separat g function the coordinates of erroneously class f ed patterns The steps included in the f gu e cor respond to several hund ed steps of the ctual computing proce dure

(c) demonstrates that the separa tion achieved in step 3 was not optimal A safety margin ± is introduced, and the process continues until the ptimal solution, (4) is found

(d) s an example of pplication and testing f the discriminant function defined in step (4) T cases, not included (A) or (B) ha e been analyzed and classif ed one into each category The results of the nal) is process is equal to the normal distance $(+D_1 \; -D)$ t the separating line (hyperplane)

that the final set of weight factors will lagerly be determined by the patterns which are most similar to those of the opposite group If differences between A and B are large there will be a large number of different sets of weight factors achieving a separation To find an optimal solution a safety margin τ is introduced, and the training procedure *is then continued until* all patterns in A and B are at a distance of ± from the hyperplane Cf Fig 10 c.

The weight *factors defining* the optimal hyperplane also indicate the relative importance of the corresponding pattern component for separation. $\frac{w_1^2}{N}, \frac{w_2^2}{N}$ are proportional to the contribution of x_1 x_2 to distinguish between A and B

Application

Caceres *(1962)* has reported some results with ECTP from a pilot study including electrocardiographic data. A training program of a different class (mode seeking) has

Linear discriminant functions can also be designed by other methods than the ECTP e.g. by postulating gaussian distributions and defining midplanes between category means.

Statistical methods

Conventional statistical methods were used for calculation of the arithmetic mean ($\bar{x}$) the standard error of the mean (S.E.) the standard deviation of the mean (S D) the variation coefficient of a single determination, and for regression and correlation analysis.

Differences between mean values were tested with Student's t-test. The degree of probability was expressed in standard terms of differences or probably significant at the 1 to 5 per cent level, significant at the 0 1 to 1 per cent level and "highly significant" below the 0 1 per cent level.

Comment Parametric methods have been applied also to ECG amplitude data which may have introduced occasional errors due to skewed distributions. Intragroup amplitude variability has generally been expressed in terms of range rather than as standard deviation.

It has been shown by among others Grewin (1948) Simonson (1962) and Draper et al (1964) that most ECG amplitude data are not normally distributed This is true for normal materials and is still more obvious in various pathological groups

An unbiased comparison of groups based on a large number of amplitude measurements sufficient to give an adequate representation of the total, three-dimensional ECG cycle will theoretically be effected by the ECTP Pipberger and Stallman (1964) have demonstrated that representation of the P QRS and ST T segment by 8/8 dipole vectors is adequate in a general clinical system for computer ECG diagnosis and a 75 dimensional pattern is easily processed by the ECTP The quantitative evaluation of the relative importance of each variable is theoretically particularly useful in the analysis of small materials not normally distributed

The individual distance to the separating hyperplane a simple numerical value, is a function of the weight factors and a large number of measurements If it can be demonstrated that this distance is clinically significant linear discriminant functions provide means of effective data reduction An additional advantage applied to linear discriminant functions the fact that each factor in the weight vector is associated with one particular amplitude measurement greatly facilitates comparison of results of ECTP with results of other analysis methods.

A list of theoretical disadvantages would include

1 A separating hyperplane may be found that is a function of (from a clinical point of view) irrelevant differences between the two groups in the training set

2 ECTP does not allow *any* errors in classification.

A large number of dimensions in relation to the number of individuals in the training set increases the degree of probability that a separating function will be found (Cf Fig 9) This fact in combination with the prerequisite that no errors are tolerated may lead to definition of weight factors which give a distorted representation of the real difference between the two groups No satisfactory mathematical method has been defined to estimate the effect of various degrees of overlapping The mathematical design of the ECTP also implies that individuals closest to the hyperplane separating the two groups largely determine the weight vector Outlying cases with characteristics similar to those of the opposite group may thus distort the results of a comparison.

These disadvantages obviously must be considered.

The program incorporates one feature to minimize the risk of distorted results the introduction of a safety margin

The validity of the weight vectors has been tested in three ways

1 Weight vector components have been compared to the actual distributions of the corresponding variable and to data from conventional electrocardiography

2 To each ECTP run has been attached a test consisting of application of the discriminant function to comparable ECGs.

3 The validity of the distance to the separating hyperplane in quantitative diagnosis has been tested against clinical and scalar electrocardiographic data.

Table 1

Group OC. Basic occupational anthropometric and physiological data.

Subject	Age	Occupation	Height cm	Weight kg	B.S.A. m^2	Heart volume supine, ml	Max. V_{O_2} lit./min.	Max. V_{O_2} ml/kg
5	52	Faculty member	188	82	2.08	—	3.03	37
6	54	Manual lab.	169	79	1.89	820	2.71	35
9	52	Lawyer	181	74	1.95	880	2.92	49
10	55	Faculty member	185	72	1.91	900	3.77	52
11	54	Merchant	169	73	1.83	750	3.02	41
12	55	Merchant	184	83	2.06	840	3.50	42
13	56	Faculty member	170	69	1.80	680	3.01	44
14	55	Teacher	179	97	2.10	1040	4.25	44
15	56	Army officer	167	71	1.80	850	3.01	42
16	47	Salesman	171	81	1.94	930	3.07	58
17	52	Mechanic	176	63	1.87	560	2.33	37
18	52	Salesman	168	64	1.72	630	2.08	32
19	51	Truck driver	190	92	2.22	970	3.39	37
20	45	Office employee	167	69	1.77	790	2.22	32
21	49	Engineer	187	84	2.10	840	3.68	44
22	50	Foreman	182	81	2.03	—	2.81	35
$\bar{X}$ ± S.E.	52.1 ± 0.8		177.1 ± 2.1	77.1 ± 2.4	1.95 ± 0.04	820 ± 35	3.05 ± 0.15	39.1 ± 1.3
S.D.	3.1		8.3	9.5	0.15	130	0.61	5.2

Faculty member denotes member of the faculty of GCI (Stockholm School of Physical Education)

Section II

CHAPTER VI

THE FRANK LEAD ELECTROCARDIOGRAM IN YOUNG AND MIDDLE AGED MALE CONTROLS DURING LIGHT MODERATE, AND MAXIMAL EXERCISE

by

G Blomqvist and *J Stenberg*

Most studies of the ECG response exercise in normal subjects have been essentially qualitative and designed to estimate primarily the frequency of false positives i e ischemic changes in normal subjects There are few truly quantitative investigations including detailed measurements of ECG amplitudes durations etc. at multiple work load levels.

The main objective of the present study was to examine quantitative ECG changes against a background of basic physiological data and to compare the ECG response to exercise in young and middle aged men, primarily at work loads corresponding to 30 60 and 100 per cent of individual aerobic capacity

Material

Group OC Middle aged men

Group OC included 16 men, mean age 52 1 years, range 45 to 56 Individual occupational and anthropometric data are presented in Table I

Reasons for exclusion from the material were a medical history or findings at physical examination suggesting cardiovascular or chronic pulmonary disease, any symptom or sign indicating an acute infection or any other current disease at the time of the examination, a diastolic blood pressure exceeding 95 mm Hg, findings in a frontal and lateral chest roentgenogram judged to be abnormal by a roentgenologist (upper heart volume limit [standing] 500 ml/m^2 B.S.A.) or laboratory findings of anemia (hemoglobin < 13 0 g/100 ml) glucosuria or proteinuria.

Group YC Young men

All members of Group YC were students who had volunteered to take part in a study on the metabolic and hemodynamic effects of a short-term physical training program. (Ekblom, to be published) It was originally planned to study also the effect of physical training on the ECG but the ECG study could not be repeated until the subjects for at least two months had reverted to their habitual level of physical activity Two subjects were excluded because no repeat study

Table II

Group Y C Basic anthropometric and physiological data

Subject	Age	Height cm	Weight kg	B.S.A. m²	Heart volume supine, ml	Max. V_{O_2} lit./min I	Max. V_{O_2} lit./min II	Max. V_{O_2} ml/kg/min. I	Max. V_{O_2} ml/kg/min. II
51	26	179	74	1.92	700	3.58	3.19	48	43
52	25	175	81	1.98	890	3.41	3.91	42	49
53	27	181	65	1.83	800	3.51	3.33	51	54
54	23	174	63	1.76	710	3.29	3.78	52	60
55	23	177	61	1.76	740	3.41	3.36	56	55
56	21	189	67	1.92	640	3.78	4.10	56	61
57	21	183	75	1.96	730	3.38	3.12	45	42
59	25	177	62	1.77	670	3.53	3.53	57	57
60	22	179	65	1.82	660	3.37	3.06	52	47
61	24	180	67	1.85	830	3.49	3.20	52	48
62	23	183	76	1.99	760	3.38	3.38	44	50
$\bar{X}$ ± S.E.	24.1 ± 0.5	179.9 ± 1.3	68.7 ± 2.0	1.87 ± 0.03	739 ± 23	3.47 ± 0.13	3.51 ± 0.11	50.7 ± 1.6	51.5 ± 1.9
S.D.	1.8	4.1	6.6	0.09	76	0.42	0.36	5.2	6.4

could be carried out. No subject in this group presented significant clinical findings

Anthropometric data are given in Table II

Methods

Electrocardiographic methods have been described Section I

Heart volume (ml) in the prone position was determined from frontal and sagittal roentgenograms according to Larsson and Kjellberg (1948) Leg exercise was performed in sitting position on an electrically braked *Krogh bicycle ergometer* with a pedal frequency of 50 rev/min In a few experiments a mechanically braked bicycle ergometer (Monark) was used *Heart rates* were calculated from ECGs A minimum of 12 R R intervals were measured.

Blood pressures were determined with the subject in supine position after 10 to 15 min. rest. A mercury manometer with a 13 cm cuff was used. Systolic and fifth phase diastolic pressures were read to the nearest 5 mm Hg

Lactate concentrations were determined in arterialized finger tip blood (the hand was prewarmed in water of 40 to 45 centigrades) according to Ström s modification (1949) of Barker s and Summerson s method The standard error of a single determination was 2 5 per cent (Saltin 1964) The mean value of a double determination was used in the calculations Samples were drawn during the last usually the sixth minute of work. At maximal loads further samples were drawn at 1 and 3 minutes after work to establish the peak concentration (Åstrand 1960)

The peak value was used instead of the mean value if the difference between determinations exceeded 5 per cent of the mean

Oxygen uptake (V_{O_2}) Expired air was collected in Douglas bags and measured in a balanced spirometer Gas samples were analyzed by Haldane technic.

Maximal Aerobic Capacity and Relative Work Load

External work loads at the maximal level were determined according to Åstrand and Saltin (1961) Heart rates during the sixth minute of exercise at 600 and/or 900 kpm/min. were employed to calculate a work load that by approximately 20 per cent exceeded the maximal level predicted from a nomogram (Åstrand 1960) A duration of maximal work of 5 to 6 minutes was considered desirable and was also achieved in all experiments, occasionally by minor adjustments (± 150 kpm/min) after 2 minutes at the calculated level if it appeared likely that the selected load would not exhaust the subject within 5 to 6 minutes, or exhaust him sooner than within 4 to 5 minutes.

V_{O_2} at the maximal level was determined from 2 samples of expired air If the difference between samples exceeded 0 08 l/min. the highest value was used in the analyses, if not, the mean value 0 08 l/min. represents the 1 per cent confidence limit for agreement between two successive samples at submaximal loads (Åstrand 1960)

The external load was increased at a repeat maximal load experiment in 9 subjects in Group OC. All had reached arterial lactate levels above 100 mg per cent at the first experiment. The mean V_{O_2} difference was —0 08 ± 0 07 l/min. S D 0 19

Both groups were studied at work loads corresponding to 30 60, and 100 per cent of aerobic capacity Individual relative work loads were determined from V_{O_2} and expressed as per cent of maximal V_{O_2} V_{O_2} va

Tabl III

Heart at elat i (% of maximal V O$_2$) and xl rnal (kpm/min) ork load and arterial lactat (mg per c nt) at vario s level Groups OC and YC Mean value S.E. and S.D

	Rest I	Rest II	300 kpm/min		450 kpm/min		600 kpm/min		900 kpm/min	
	HR	HR	%	HR	%	HR	%	HR	%	HR
OC N = 16	71 6±3 1 12.5	72 9±3.7 14 7	32 1±1 6 6.5	94 8±3 2 12 7	—	—	55 8±2 9 11 4	130 7±4 5 18.0	77 1±3.5 14.1	148 6±5 5 21 9
YC N = 11	73 1±2.2 7 1	71 1±3.1 10.1	27 1±0 9 2 9	96 5±2 4 8 0	36 1±0 4 1 1	107.0± 7 8 9	45.5±1 4 4 6	114 6±2.5 8.4	62 6±0.7 2.2	146 0±5 1 9 1

	Estimated 60 per cent				Maximal load I			Maximal load II		
	Ext load	%	HR	Lactate	Ext load	HR	Lactate	Ext load	HR	Lactate
OC N = 16	715± 12 170	61 1±1 4 5.5	133 1±3 6 14 3	31.1±3 9 14.4	1 303±67 260	177 9±2 0 8.0	109 3±5 8 22.4	1,388±67 260	176.5±3.5 13 6	116.5±6.2 24 6
YC N = 11	900 —	62.5±1 9 6 3	146 0±5 1 16 8	36 5±3.1 13 9	1,691±43 144	185 6±2.1 7.9	132.4±7.2 21 5	1 636±32 105	188 9±2.5 8 3	131 9±4 5 13 6

lues of 0 96, 1.25 and 1 58 l/min were used to calculate relative loads at 300 450 and 600 kpm/min These values were derived from a combination of all male normal groups studied by Åstrand and Åstrand (I Åstrand 1960)

Actual V_{O_2} measurements were made in all subjects in Group OC at the 60 per cent level Eleven determinations were made at 900 kpm/min The mean value was 2 22 ± 0 03 l/min S.D 0 10 which did not differ significantly from a mean value of 2 19 *l/min. reported by Astrand (1960) from a corresponding group examined with identical* methods Four determinations were made at 600 kpm/min in Group OC. The mean value was 1 53 l/min., range 1 48 to 1 62.

External work loads were individually calculated to yield a V_{O_2} corresponding to 60 per cent of maximal aerobic capacity in Group OC. Group YC was *much more* homogeneous. Only standard loads were used 900 kpm/min. closely corresponded to a *relative load of 60* per cent *(Table III)*

No attempt was made to calculate individual external work loads at the 30 per cent level A standard load of 300 kpm/min. was used Calculations based on estimated V_{O_2} demonstrated a 5 per cent difference in relative load between the groups, 32 4 per cent in Group OC versus 27 4 per cent in Group YC. The difference was probably significant. The small difference in relative load at the 60 per cent level, 61 4 versus *62 5 per cent, was not significant.*

Procedure

Each subject was studied twice The interval between examinations averaged (median) 14 days in group OC, range 2 to 98 days. In fifteen of the sixteen subjects experiment II was carried out within 30 days. The *average interval was 141 days in group YC,* range 120 to 221 days

The procedure was identical at both examinations, excluding a medical history interview and a complete physical examination at the initial study The subjects reported to the laboratory in the afternoon or in the evening They had not engaged in *any heavy physical activity earlier during the* day or had any meal within 2 hours prior to the examination No smoking was allowed during the *last* hour Room temperature *ranged between 17 and 23 centigrades. The* humidity was not recorded

Height and weight were measured at the beginning of the experiment. A physical examination starting with blood pressure recording followed a medical history interview Frank lead ECG electrodes were *applied. ECG at rest* was recorded with the subject in sitting position.

The sequence of work loads was 300 600, *and 900 kpm/min and maximal work at* experiment I in group OC and 600 60 per cent of maximal and maximal work in experiment *II* Corresponding *loads* in group YC were 450 900 and maximal at experiment I and 300, 600 900 and maximal load at experiment II

The duration of work was 6 minutes at each submaximal level, and 5 to 6 minutes at the maximal level Rest periods of 10 minutes were interspersed at loads below 50 per cent of maximal capacity and of 15 minutes at higher loads.

Work at the maximal level was *immediately* preceded by a warm-up period of 2 to 3 minutes at a load corresponding to 60 per cent of maximum.

The ECG was monitored on an oscilloscope A 45-second tape recording of

the Frank lead ECG was made during the last minute of work, and immediately after and 3 minutes after work. Scalar ECGs (CH_2, CH_4, CH_6) were recorded at 2 minute intervals during work and immediately and 3 minutes after work in Group OC.

Results

Symptoms

The procedure did not in any case provoke chest pain or any other remarkable symptom, nor did any subject complain of unpleasant after-effects.

Anthropometric and Physiological Data

Anthropometric data, heart volumes, and maximal V_{O_2} are presented in Tables I and II. There was essentially no difference in height between the young and middle-aged men, but the men in Group OC were heavier. The difference between the mean values 8.4 kg., was probably significant.

The mean heart volume of Group OC was higher 820 versus 739 ml., but intragroup variation was large, and the difference was not significant. Absolute and relative maximal V_{O_2} values were significantly lower in Group OC, 3.05 and 39.4 versus 3.47 l/min. and 50.7 ml/kg/min.

Mean heart rates and lactate levels at the various work load levels are given in Table III. There were no group differences in heart rate during work at equal external work loads. The mean maximal heart rate was significantly lower in group OC by 12 beats per minute, 177 versus 189. A corresponding difference (probably significant) was found at the 60 per cent level, 146 versus 133.

Lactate levels were slightly higher in Group YC at the 60 per cent and at the maximal level. Intragroup variability was large, and the differences were not significant.

Intragroup variability was smaller in Group YC with regard to the variables listed in Table III except at the 60 per cent level. Work loads had been individually determined for the subjects in Group OC. Standard deviations for heart rate and per cent of maximal V_{O_2} were somewhat lower than in Group YC. The variation in heart rate within Group OC was also smaller at the 60 per cent level than at comparable standard loads of 600 and 900 kpm/min.

ECG Analysis

The following aspects have been considered in the analysis of the ECG during exercise in the two groups

1 QRS and ST T changes at loads corresponding to 30 60 and 100 per cent of individual aerobic capacity evaluated by inter and intragroup comparisons of

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Fig. 11 Group mean QRS and ST T 8/8 vector components at rest and during exercise at levels corresponding to 30, 60, and 100 per cent of individual aerobic capacity Young and middle-aged control

Bold-faced figures refer to 8/8 QRS and ST T vector components Top lead X, middle Y bottom Z. Vertical scale divisions correspond to 0.1 mV The scale is expanded twice for ST T in comparison to QRS

Group YC (young controls, N = 11) is represented by open circles, Group OC (N = 16) by filled circles The sequence of ECG amplitudes at rest and during work is represented by the four circles within each 1/8 vector column (in order from left to right, rest, 30, 60, and 100 per cent of maximal aerobic capacity)

1	2	3	4	5	6	7	8

1	2	3	4	5	6	7	8

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ECG Analysis

The following aspects have been considered in the analysis of the ECG during exercise in the two groups:

1. QRS and ST-T changes at loads corresponding to 30, 60, and 100 per cent of individual aerobic capacity evaluated by inter- and intragroup comparisons of

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Fig. 11. Group mean QRS and ST-T 1/8 vector components at rest and during exercise at levels corresponding to 30, 60, and 100 per cent of individual aerobic capacity. Young and middle-aged compared.

Bold faced figures refer to 1/8 QRS and ST-T vector components. Top: lead X, middle: Y, bottom: Z. Vertical scale divisions correspond to 0.1 mV. The scale is expanded twice for ST-T in comparison to QRS.

Group YC (young controls, N = 11) is represented by open circles, Group OC (N = 16) by filled circles. The sequence of ECG amplitudes at rest and during work is represented by the four circles within each 1/8 vector column (in order from left to right: rest, 30, 60, and 100 per cent of maximal aerobic capacity).

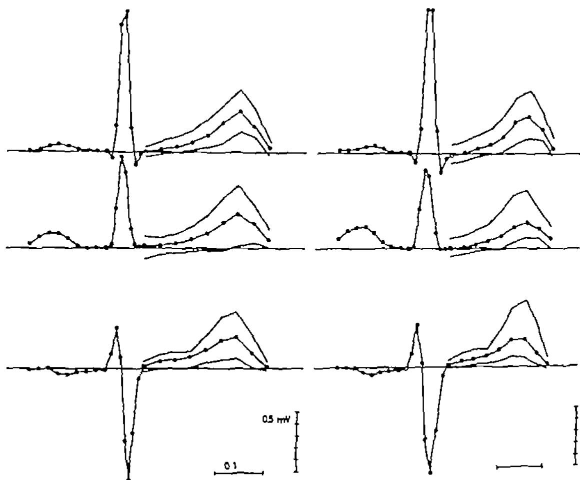


Fig 12 Group mean P QRS and ST T 8/8 vector components and ST T ang in middle-aged men (Group OC) at rest and during exercise at loads corresponding to 30 60 and 100 per cent of individual maximal aerobic capacity

(a) ECG at rest and during work at the 30 per cent level. Heart rates 75 and 95

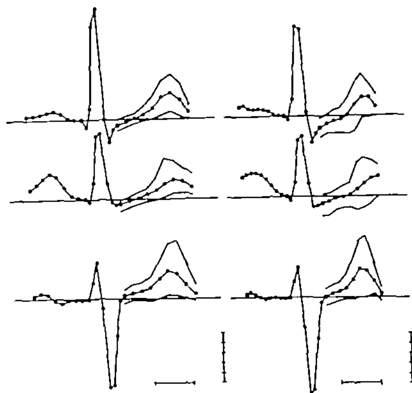
amplitudes in individual orthogonal leads

2. Relationships between ST junction amplitudes and heart rate P and T P amplitudes
3. QRS and Q T duration changes
4. Inter and intragroup QRS and ST T differences evaluated by means of the linear pattern classification program described in Chapter V

QRS and ST T amplitude data are presented in Fig. 11 12, and 13

Intergroup differences at rest The maximal QRS vector QRS 4, had larger posterior and inferior components in Group YC, but intragroup variation was large and the differences were not significant Mean X, Y and Z components were 1 37 1 06 and —0 85 mV in Group YC, and 1 17 0 64 and —0 54 mV in Group OC

The Y component of QRS 3 was significantly larger in Group YC, 1.27 versus 0 73 mV Other QRS differences were relatively small and not significant, except Y and Z



(b) ECG during ork at the 60 per cent and the maximal level Heart rates 133 and 177

lead differences in J amplitude with larger positive amplitudes in Group YC. These differences were probably significant. Similar differences during the early part of the ST T segment in leads Y and Z were also probably significant. Differences in maximal T amplitude (ST T 6) were not significant. Mean X, Y and Z components were 0 36, 0 36 and 0 32 mV in Group YC, and 0.33 0.28, and 0.27 mV Group OC.

QRS and ST T hanges ith increasing rk load

QRS hanges. QRS changes with increasing work load showed essentially the same pattern in both groups (Fig. 11) with deviation of initial vectors QRS 1 to 4 upwards and to the right as evidenced by decreasing positive X and Y and increasing negative Y and Z voltages. Terminal vectors QRS 5 to 8 also showed a change upwards and to the right, but a decrease in negative Z voltages indicating an anterior displacement in comparison to the resting records.

Intragroup variability was large, but rightward displacements from rest to maximal work in QRS vectors 4, 5 and 6, upward displacements of QRS 6 were all highly significant or significant in group YC. Group OC showed significant or highly significant

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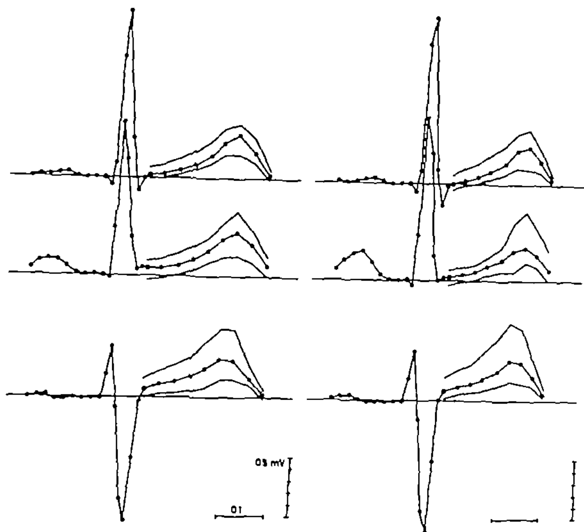
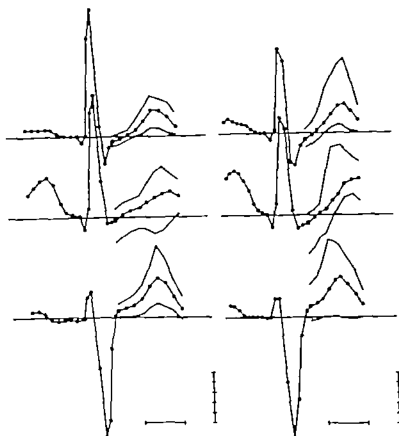


Fig 13 Group mean P QRS and ST T 8/8 vector components and ST T ang young men (Group YC) at rest and during exercise at loads corresponding to 30 60 and 100 per cent of individual aerobic capacity

(a) ECG at rest and during work at the 30 per cent level Heart rates 73 and 97

rightward displacement of QRS 6 and 7 upward displacement of QRS 7 anterior displacement of QRS 1 and 6 and posterior displacement of QRS 3 and 4 (Fig 11)

QRS 8 ST J decreased gradually with increasing work loads in leads X and Y in both groups, but intragroup variability was large, and intergroup differences not significant except in lead X during maximal work. Mean X and Y J amplitudes in Group OC were 0 03 and 0 02 mV at rest, —0 02 and 0 01 at 30 percent, —0 06 and —0 05 at 60 percent, and —0.12 and —0 07 at the maximal level The range in Group OC during maximal work was —0 03 to —0 21 mV in X and —0 02 to —0 19 mV in Y Inter group differences were small in both X and Y during work at the 30 and 60 per cent levels. The mean value QRS 8 X value in Group YC was —0 07 mV during maximal



(b) ECG during ork at the 60 per cent and at the maximal level Heart rates 146 and 189

work with range of —0 14 to —0.04 mV The difference between Groups YC and OC, —0.07 versus —0 12 mV was probably significant Cf Fig. 12 and 13

Over-all QRS changes were evaluated by estimating the time integral in the three leads from the sum of the QRS 8/8 vector component amplitudes and calculating the angular change of the QRS area ector in terms of elevation (0° = left, 90° = inferior) and azimuth (0° = left, 270° = posterior) Elevation in Group OC changed from 39° at rest to 44 during maximal work, and azimuth from 312 to 288 Corresponding values for elevation in Group YC were 50 and 62° and for azimuth 324 and 303

Thus, Group YC showed a relatively more marked right axis deviation during maximal exercise. The change toward more strictly posterior mean QRS vector was of approximately the same magnitude in both groups.

ST T changes ST T changes with increasing work loads are outlined in Fig. 11 Fig. 12 and 13 display mean amplitudes and ST T ranges in both groups

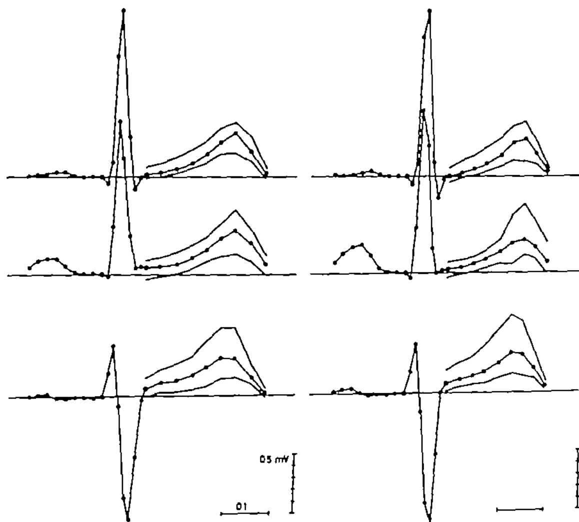


Fig 13 Group mean P QRS and ST T 8/8 vector components and ST T angle in young men (Group YC) at rest and during exercise at loads corresponding to 30 60 and 100 per cent of individual aerobic capacity

(a) ECG at rest and during work at the 30 per cent level. Heart rates 73 and 97

rightward displacement of QRS 6 and 7 upward displacement of QRS 7 anterior displacement of QRS 1 and 6 and posterior displacement of QRS 3 and 4 (Fig 11)

QRS 8 ST J decreased gradually with increasing work loads in leads X and Y in both groups, but intragroup variability was large and intergroup differences not significant except in lead X during maximal work. Mean X and Y J amplitudes in Group OC were 0 03 and 0 02 mV at rest, —0 02 and 0 01 at 30 percent, —0 06 and —0 05 at 60 percent, and —0 12 and —0 07 at the maximal level The range in Group OC during maximal work was —0 03 to —0 21 mV in X and —0 02 to —0 19 mV in Y Intergroup differences were small in both X and Y during work at the 30 and 60 per cent levels The mean value QRS 8 X value in Group YC was —0.07 mV during maximal

Six subjects developed segmental ST depressions during maximal work. The ST segment was normalized immediately after work in one subject, and 3 minutes after work in two. Horizontal ST depression of 0.05 to 0.10 mV was still present 3 minutes after work in 3 men. All 3 men with transient ischemic ST changes demonstrated the same pattern at both series at maximal loads. One of the men with changes still present 3 minutes after work also presented the same pattern at both examinations. Of the two remaining subjects, one had nonremarkable ECG response at the first examination but showed a 0.05 mV ischemic depression 3 minutes after work at the repeated test, and the other had 0.15 mV horizontal depression 3 min. after work at the first maximal test but no ST depression after a slightly higher load at the second examination.

The absolute amplitude of the horizontal ST depression was smaller in the X lead than in CH 4 and 6. The ST depression exceeded 0.10 mV in CH leads in 8 ECGs recorded during or after exercise at the maximal load. Six of these subjects also had horizontal ST depression of 0.10 mV or more in lead X, but in 2 cases was the amplitude only 0.04 mV.

In summary Group OC showed larger relative X lead ST-T amplitude changes with increasing work loads than did group YC. Intergroup differences were definitely larger at the maximal than at the 30 or 60 per cent level in lead X. Y lead amplitudes were difficult to evaluate due to marked changes in T-P level. Z lead intergroup differences were similar at rest and during work.

QRS and Q-T Duration

Few reports are available on QRS-T etc. durations in normal subjects at heart rates above 150. Group means and standard deviations were calculated for P-R, QRS, ST and Q-T durations at the work loads listed in Table III.

The wave recognition program gave preference to the end of the T wave over the beginning of the P wave at heart rates above 160—170 where the waves are superimposed. No analysis of P-R durations will thus be reported.

The duration of the ST segment showed a linear relationship to the R-R interval in both groups with a slightly longer duration in Group OC. The difference was not significant. The coefficient of variation was consistently 2 to 5 times larger than for any other time measurement, or 20 to 25 per cent versus 5 to 10. No detailed analysis has been attempted.

QRS Duration The QRS duration at rest was slightly shorter in Group OC, 86.0 ± 1.5 S.D. 6.2 versus 90.0 ± 1.6, S.D. 5.5 msec. in group YC. The difference was not significant.

The duration increased gradually during exercise at progressively heavier loads in both groups. A mean value of 92.5 ± 2.3 S.D. 5.4 msec., was found in Group OC during maximal work. The corresponding value in Group YC was 95.3 ± [illegible].5 S.D. 7.0 msec. The difference between rest and maximal work was probably significant in Group OC. A prolongation was noted also in records immediately after and 3 minutes after exercise at loads at the 60 per cent level and above. The QRS duration returned to resting values within 5 minutes after exercise at lower loads.

Q-T Duration The mean Q-T duration was as a whole slightly shorter in Group YC but differences were small and non-significant. Group mean Q-T variation with R-R

Fig 11 demonstrates that the change from resting amplitudes followed the same pattern in both groups in all three leads at the 30 and 60 per cent levels but that there were gross intergroup differences at the maximal level It is also evident from Fig 11 that there were large intergroup differences with regard to ST T 8 in Y and Z at the maximal level and that these discrepancies were parallelled by differences in Y and Z components of ST T vectors 3 through 7 There were no significant intergroup ST T 8 differences in lead X ST T 8 amplitudes closely corresponded to the level of the T P segment at the beginning of the P wave at the 60 per cent and the maximal level

Intragroup comparisons showed no significant deviations from the resting level at any load in lead X in Group YC. ST T 1 showed the largest relative change from 0 04 mV at rest to —0 07 mV during maximal exercise Peak T amplitude decreased from 0 36 mV at rest to 0 28 mV during maximal work. Y lead changes were difficult to evaluate except during the early part of the ST T segment due to the large change in T P level The decrease in ST T 2 Y amplitude from the resting level was probably significant during work at the 60 per cent level, and the differences between rest and maximal work were also probably significant for both ST T 1 and 2

YC intragroup differences in lead Z were not significant. Intragroup variability was smaller in Group OC at each work load with regard to most ST T items Differences in the X lead between ST T 1 2 and 3 amplitudes at rest and during work at the 60 per cent level were significant The large differences in absolute amplitudes of X components of ST T vectors 1 through 6 between rest and maximal work were also highly significant (1 to 4) or significant (5 to 6) ST T 1 2, and 3 amplitudes all became negative during maximal work, i.e. a ST depression including approximately 3/4 of the total ST segment duration. The mean amplitude of ST T 3 was —0 04 mV with a range of —0 17 to 0 08 mV The mean peak T amplitude decreased from 0.33 mV at rest to 0 18 mV during maximal work (range —0 02 to 0 41 mV) Y lead amplitudes also showed larger intragroup differences between different loads in Group OC than in YC. ST T 2 and 6 differences between rest and the 60 per cent load were probably significant. Differences between rest and maximal work were highly significant for ST T 1 through 6. Lead Z amplitudes showed little change with increasing work loads also in Group OC. No differences were significant.

Intergroup comparisons at the maximal level between X lead amplitudes demonstrated a probably significant difference for ST T 1 significant differences for ST T 2, 3 and 5 and a highly significant difference for ST T 4 The differences in maximal T amplitude, ST T 6, was not significant Y lead differences were not statistically evaluated. Intergroup differences in Z lead amplitudes during work were of the same magnitude as at rest and significant or probably significant at all work load levels for ST T 1 through 6.

CH leads in group OC Analysis of CH leads revealed no ST T changes at rest in any subject. During work at loads higher than 300 kpm/min. some ST J depression was generally found. Only 2 subjects failed to show any measureable junctional depression (0 05 mV or more) at the 600 level, and only 1 had no J depression at 900 All had some J depression during maximal work.

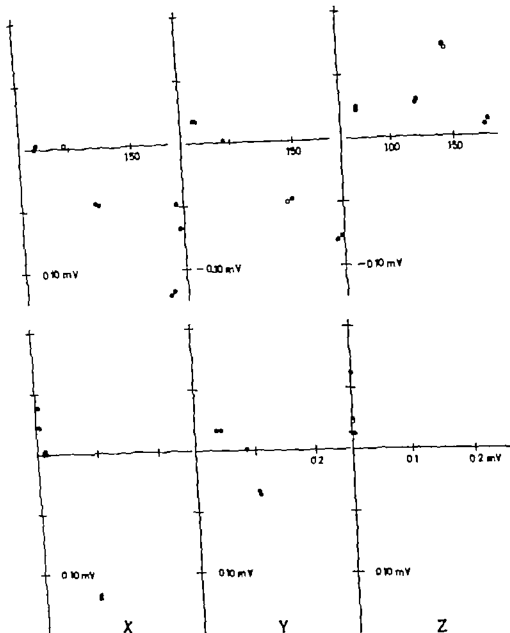


Fig. 15 Relationship between Group mean J amplitude and ( ) heart rate (b) T-P amplitude at rest and during exercise at ... levels in control subjects.

Ordinate J amplitude Abscissa ( ) Heart rate, (b) T-P amplitude. Filled circles Group OC, open circles Group YC. The levels of exercise are listed in Table III

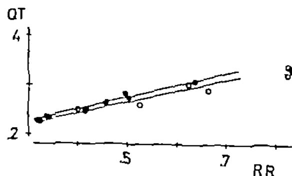


Fig 14 Correlation between group mean R R interval and Q T duration during exercise
Filled circles represent group means at various levels (Table III) of exercise in Group OC, open circles Group YC.

interval during exercise is demonstrated in Fig 14 The Q T *duration decreased from* 333 0 ± 5 3 S D 24 5 msec. at rest to 233 7 ± 2 8 S D 6.3 msec during maximal exercise in Group YC. Corresponding values in Group OC were 342 4 ± 3 1 S.D 18 4 *and 238 5 ± 2.5 S.D 15 5 msec. If* resting values were included, the slope appeared slightly curvelinear An analysis limited to Q-T durations during exercise showed a linear relationship and high coefficients of correlation in both groups, $r = 0.95$ in Group YC and 0 97 in Group OC, and the following regression lines (Q-T and R R in msec)

Group OC Q T = 130 + 0 29 R R
Group YC Q-T = 140 + 0 25 R R

ST Junction

Mean ST J amplitudes in leads X Y and Z in both groups at rest and during exercise at the levels listed in Table III are demonstrated in Fig 15 Ranges at rest and during exercise at 30 60 and 100 per cent of aerobic capacity are illustrated in Fig 12—13 Group mean J amplitudes differed significantly from the zero amplitude of the P R segment immediately before the beginning of QRS in most recordings in both groups Exceptions were X lead amplitudes at rest and at work loads below 900 kpm/min., and Y amplitude at 450 kpm/min. in Group YC, and X, Y and Z amplitudes at rest Y amplitude at 300 kpm/min. and Z amplitude during maximal work in Group OC.

J amplitudes in lead Z were positive, i.e. an anterior orientation of the J vector at rest and at all work loads *in both groups* J amplitudes were significantly higher in Group YC except in one of the resting recordings Group YC had a J vector directed to the left and downwards at rest. The direc *tion gradually changed to rightwards-up*wards with increasing heart rates. Resting values in lead X were close to zero in Group OC. There were no significant differences between Groups OC and YC in lead X at *heart rates below 150 The difference during* maximal exercise was probably significant. The positive Y amplitude at rest was larger in Group YC.

The relationship between mean J ampli tude and heart rate is demonstrated *in fig* 15 Lead X amplitude in Group OC form a hyperbolic regression line A parabolic re gression line gave a closer fit in Group YC. Lead Y amplitudes displayed a hyperbolic relationship in both groups, *while lead Z* amplitudes showed little variation with heart rate

Curve forms were *fitted* graphically and *least* squares were calculated for various alternatives. *The number of points was rela* tively small and the scatter relatively wide with regard to *most relationships No attempt* was made to define regression equations

The intersection between the approxima *tive regression lines* and the abscissa estimates

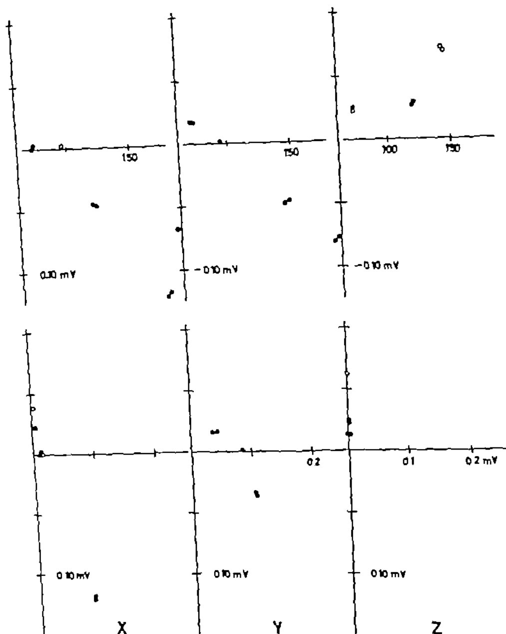


Fig. 13. Relationship between Group mean J amplitude and ( ) heart rate (b) T P amplitude at [illegible] and during exercise at [illegible] levels in control subjects.

Ordinate: J amplitude. Abscissa: ( ) Heart rate, (b) T P amplitude. Filled circles Group OC, open circles Group YC. The levels of exercise are listed in Table III.

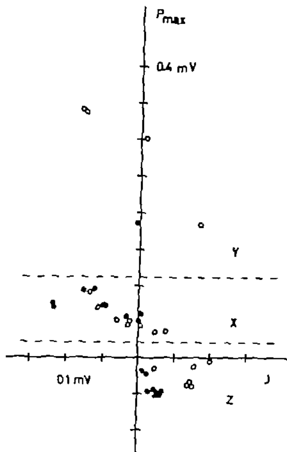


Fig 16 Relationship between group mean maximal P and J amplitudes as measured during exercise in control subjects

Group OC represented by filled, Group YC by open circles Levels of exercise listed in Table III

at which heart rates mean J amplitudes in lead X and Y changed from positive to negative This occurred at a rate of 75 in lead X and 85 in lead Y in Group OC Corresponding values for Group YC were 95 and 110

The relationship between T P segment and J amplitudes (Fig 15) showed similarities to that between heart rate and J amplitudes. However, distributions suggested parabolic regression equations in leads X and Y in both groups J amplitudes in lead Z showed a small increase with increasing T-P amplitudes in Group YC.

Fig 16 shows the relationship between maximal P and J amplitude. The scatter was very wide in both groups in all leads with the exception of lead X amplitudes in Group YC which suggested a hyperbolic regression line. Maximal P amplitude is equal to maximal P + T due to the definition of the base line.

ECG changes with increasing work loads evaluated by a linear pattern classification procedure

The linear error-correcting training procedure (ECTP) described in Chapter V was applied to data from the two control groups in inter and intra group comparisons. Intra group comparisons were based on sets of 75 measurements, X, Y and Z amplitudes for P 0 and 8/8 P QRS and ST T vectors. Intergroup comparisons were limited to terminal QRS and ST T vectors, but included a complete series of four ECGs (at rest, during exercise immediately after and 3 minutes after exercise) each represented by QRS vectors 6, 7 and 8 and ST T vectors 1 through 6, or together 108 amplitudes values.

The ECTP generates a discriminant function including a set of weight factors, one for each amplitude measurement. From the logic of the ECTP it follows that each weight factor also measures the relative importance of the corresponding variable in defining the percentage of the total difference attributed to the associated variable.

Disadvantages of the ECTP have been discussed in Chapter V It was pointed out that the linear discriminant function must be validated, and that this could be done by observing the results of the classification procedure in comparable cases with known

classification. A large number of ECTP comparisons were carried out. Two will be reported in some detail

Intra-group comparison The ECTP defined discriminant functions, i.e. separating hyperplanes, in comparisons between the ECG at rest and during work at the 30 per cent level during work at the 30 and 60 per cent level, and at the 60 per cent and maximal level. No separation was achieved in comparisons between the ECGs at rest in experiments I and II nor between repeated tests at 600 kpm/min. in Group OC and at 900 kpm/min. in Group YC. Only comparisons between 60 per cent and maximal loads generated valid discriminant functions as judged from the classification results in test groups.

A separating hyperplane with a safety margin, of ± 10 units was defined in comparison between ECGs during work at the 60 per cent and at the maximal level in Group OC. The discriminant function was tested against ECGs recorded in Group OC at the maximal load and at 900 kpm/min. in experiment I. All were correctly classified.

Forty-eight per cent of the total difference between ECGs during work at these two loads was due to ST T segment changes, 33 per cent to QRS and 19 per cent to P changes

ECGs recorded at the 60 per cent level were characterized by positive distances to the separating hyperplane The combination of positive amplitude values and positive weight factor contributed to classification of the ECG as recorded at the 60 per cent level

The largest weight factor was found for the X component of ST T vector 5 + 31 and the second largest for the X component of ST T 4 + 25 Fig. 21 shows that the mean X amplitude of ST T 5 was approximately twice as high during work at the 60 per cent level as at the maximal load, and that the mean X amplitude for ST T 4 was 0.1 mV at 60 per cent but close to zero during maximal work. Amplitude differences in ST T X components 4 and 5 defined 16 per cent of the total difference. The proportion accounted for by all ST T X components was 26 per cent and by ST T Y components 18 per cent including 7 per cent by ST T 8 i.e. the change in T P level. ST T Z components were much less important. Their total contribution amounted to only 2 per cent. Fig 21 also shows that only minor changes occurred in the Z lead.

A corresponding comparison in Group YC between the 60 per cent and maximal load attributed 37 per cent of the total difference to ST T changes. A major part, 14 per cent, was due to changes in ST T 8 i.e. T P level. QRS changes accounted for 35 per cent, and P changes for 27 per cent, including 14 per cent contributed by P 0 Change in T P level thus defined at least 28 per cent of the total difference.

Inter group comparisons A valid discriminant function was found only for the ECG series at the maximal level. Test series from both groups were classified correctly except in one case that was defined as borderline. Group differences at rest accounted for 2 per cent of the total difference. Differences during exercise defined 43 per cent, immediately after 16, and 3 min. after exercise 15 per cent of the total difference. Amplitudes in the X lead during exercise accounted for 30 per cent of the total intergroup difference. The largest weight factors were those of the X components of QRS 8 or the ST junction, and ST T 2 and 4. Cf Fig. 11

Discussion

Material and procedure

Both control groups were highly selected No attempts were made to collect control groups constituting random samples of a general city population

It was considered desirable to study the subjects primarily at three different work load levels, including maximal work. Use of standard loads would have resulted in grossly unequal steps between medium and maximal work loads both with regard to relative and external loads The range in relative load in Group OC at e g 900 kpm/min. was 57 to 100 per cent of maximal aerobic capacity External loads at the maxi mal level varied between 900 and 1800 kpm/min

The subjects in Group OC were selected to provide a control group with a wide range with respect to occupation, physical activity and work capacity The proportion of profes sional men was obviously higher than in a general city population, but subjects with heavy manual work were included The intragroup variation with regard to maximal absolute V_{O_2} amounted to ± 50 per cent of the mean and with regard to relative V_{O_2} to ± 24 per cent.

The group mean relative V_{O_2} was somewhat higher than that reported by Astrand (1958) from a series of forty four 50 to 54 year old Stockholm brewery truck drivers or 39 4 versus 33 9 ml/kg The maximal heart rate was also higher in the present series, with a mean of 177 versus 161 The mean maximal lactate value was considerably higher in Group OC, 112 versus 58 mg per cent. Heart rates at 600 kpm/min. were essentially the same, 119 and 120 but heart rate and V_{O_2} standard deviations were larger in Group OC. The heart rates at 600 and 900 kpm/min, were slightly lower but did not differ significantly from those in the normal series studied by Strandell (1964) The maximal heart rate and maximal lactate in Group OC were somewhat higher than those reported by Astrand (1960) and Strandell (1964) in corresponding series of normal subjects. These data may be interpreted to indicate that the Group OC had a higher than average physical work capacity as their mean maximal V_{O_2} was higher than that of a group of men employed with heavy manual work. The high maximal lactate level and the fact that maximal V_{O_2} did not change with increasing external loads suggest that true maximal values were achieved (Taylor et al. 1955 Astrand and Saltin 1961) and also that the subjects in this series may have pushed themselves harder than those in the series quoted.

Autopsy studies (for a review see e g Plotz 1959) suggest that a large proportion of all men above 35 years of age have some coronary artery narrowing It thus seems highly probable that group OC included subjects with significant coronary artery disease The screening procedure was only intended to eliminate cases with overt cardiovascular disease

Group YC was studied mainly to provide a background to findings in Group OC. Data on maximal V_{O_2} suggest that the group did not represent any extreme with regard to physical work capacity Relative maxi mal V_{O_2} was somewhat lower than that reported by Astrand (1960) for a group of 29 men 20 to 33 years old, 51 ml/kg versus 59

There was no difference in mean heart rate during work at 900 kpm/min or in mean maximal V_{O_2} between the two exami nations, which indicates that there was no

change in average physical work capacity in Group YC.

The significance of maximal oxygen uptake measurements as a means of characterizing physical work capacity and relationships between hemodynamic adaptations to exercise have recently been discussed by Taylor et al. (1963) Saltin (1964) and Strandell (1964) A linear or nearly linear increase in cardiac output and arterial blood pressure with an increase in V_{O_2} suggests that a work load sufficient to produce maximal oxygen uptake also places a maximal stroke work load on the myocardium, but conclusive hemodynamic data are not available.

ECG Analysis

ECG at rest Simonson (1961) has reviewed in detail sources of biological variability with respect to the ECG. He maintained that age is the most important of the constitutional variables affecting the ECG The difference between Groups YC and OC in the resting ECG conforms to general age trends with higher amplitudes and more vertical heart position in the younger group.

ST T amplitudes in Group OC were in all subjects within the normal range of the Frank lead ECG as reported by Draper et al. (1964) but the upper limit of Draper's material was exceeded for QRS 3 and 4 Subjects in Group YC also exceeded the upper range with regard to ST T amplitudes. These differences are similar to the deviations from normal values in physically active populations reported by Rautaharju (1963)

QRS change during work Kimura and Simonson (1953) studied the spatial ECG in small series of young normal subjects. QRS changes immediately after heavy submaximal work load corresponded to those observed in the present series during maximal work with a shift in vertical and backward direction and a decrease in magnitude of the mean QRS vector Changes were however less marked immediately after maximal work of short duration. Kahn and Simonson (1957) reported on a series of 35 young males studied immediately after maximal work. They found also in this series a significant backward rotation of the mean QRS vector and a small change in elevation toward a more vertical heart position.

Arvedson (1963) reported changes in the same direction in a series of normal subjects studied with Frank's lead system.

No detailed data have earlier been published on spatial QRS changes during work. There were marked similarities between the two groups in the response to increasing work loads with a few exceptions where changes were more marked during maximal work in Group YC, i e. larger negative amplitudes of terminal X components. The pattern is compatible with and effect primarily of a change in heart position V_{O_2} and ventilation were higher in Group YC which may explain differences in X components 5 6, and 7

ST T changes during work The reduction of ST T amplitudes in lead X with no change in Z amplitudes is in agreement with earlier observations on an anterior displacement of the T vector with exercise, e.g. Kahn and Simonson (1957) and also with Bengtsson (1956) findings in conventional leads in adult subjects.

The large change in T P level in the Y lead presents marked difficulties with regard to vector interpretation of ST T data. Thus, no attempt has been made to calculate change in spatial QRS-T angle. Measurement of ST T amplitudes from straight line connec

ting ST T 8 with the baseline at the end of QRS may represent a useful approximation. Application of this method to the data in Fig 11 transforms Y amplitudes into a pattern closely corresponding to that in lead X where amplitudes are much less influenced by the changes in T P level.

Recently Doan et al (1965) reported a study on the ECG after maximal exercise in 433 assymptomatic men. They concluded that the sensitivity of the maximal capacity exercise test was nine times greater than that of the double Master's two-step in eliciting ECG evidence of myocardial ischemia, but also pointed out that the significance of the finding would rest on the results of follow up studies. Clinical exercise tests including work at a maximal or near maximal level has been common practice in Sweden for many years (Sjöstrand 1951) but no follow up studies on the prognostic significance of ST T changes provoked at various work loads levels are available

The main finding in this study in the older group of a largely linear response similar to that in the young control group at work loads up to 60 per cent of aerobic capacity but much more marked changes at the maximal level suggests that caution should be exercised in the interpretation of ST changes provoked by maximal or near maximal loads

It is conceivable that a segmental ST depression provoked at a maximal load has less prognostic significance than changes at relatively light loads corresponding to a double Master's test, but that changes at a maximal load nevertheless give a better estimate of the prevalence of coronary artery disease. Six of 16, or 37 5 per cent of the men in Group OC showed horizontal ST depression of 0 5 mm or more at one or both maximal load experiments. The depression exceeded 0 9 mm in 5 subjects at the first and in 4 subjects at the second experiment. Doan et al (1965) found a similar depression in 9 cases of 32 at maximal work in the same age group. Astrand (1965) found only 3 cases of segmental depression of 1 mm or more in a random Stockholm sample of 73 men in an age group corresponding to Group OC, but exercise was not carried further than to heart rates of 150. Strandell (1964) found 3 cases of segmental ST depression exceeding 0 5 mm in a series of 14 subjects 50 to 59 years old studied at maximal or near maximal levels.

All series are small but the data reported are compatible with a steep increase in the frequency of segmental ST depression if work loads are increased from a relatively high submaximal to a maximal level

ST Junction The fact that junctional amplitudes generally were significantly different from zero is in agreement with the observation that there is no true isoelectric baseline (Chapter III)

The clinical significance of a junctional depression during and after exercise has been a matter of dispute. Sjöstrand (1950) measured the level of the ST segment 20 msec after J and found a close relationship between heart rate and ST change in normal subjects.

Robb, Marks, and Mattingly (1957) and Mattingly (1962) have reviewed the litterature and presented data to support the view that J depression is a normal response to exercise in contrast to segmental ST depression. Astrand (1965) found no correlation between J depression and coronary disease and no correlation between J depression and segmental ST depression in an 8 year follow up of a random population sample. The present series showed a strong relationship

between heart rate and J depression in both groups (Fig. 17)

J depression with increasing heart rate has been attributed to a increasing T amplitude (Lepeschkin and Surawicz 1958)

It also been held that the artial gradient is equal to zero, i.e. that P nd T areas derived by integration in any ECG lead are of equal magnitude but have opposite signs (Pipberger et al 1961) It would thus be reasonable to assume a correlation between P and J amplitude. Fig. 16 shows that there was no close relationship between P and J amplitudes in this material except in lead X in Group YC. The data presented in Fig 16 and 17 rather support Sjöstrand's iew (1950) that J depression is due to a positive after potential following the T wave However the relationship demonstrated in Fig 17 is not conclusive. Group Y had a more marked elevation of the T P level but less J depression than Group OC and also higher P amplitudes It is thus possible that the ST J depression with increasing heart rates n normal subjects results from an inter action between T and positive after-potentials

QRS D ratio Several investigators have found decrease in QRS duration with exercise in normal subjects, g. Shellong and Luderitz (1953) who employed changes i QRS duration as an important part f a cardiovascular function test.

Blackburn and Simonson (1957) ecorded simultaneous orthogonal leads in a group f 10 normal subjects before and immediately after hea y exercise of short duration. They found a highly significant mean *increase* of 4 5 msec i.e. a change in the same direction and of the same magnitude as in our series.

Blackburn and Simonson also found that QRS duration measurements in isolated conventional leads result in a significant under estimation of the total duration measured in three simultaneous orthogonal leads. Change in heart position may explain an pparent decrease in QRS duration with exercise in conventional leads

Q T Duratio Simonson et al (1962) have examined suggested formulas for the relationship between R R and Q-T intervals. They listed nine different alternatives, including Bazett s square root and Fridericia s cube root formula.

Six different methods were tested n a normal material f 960 adult men and women and compared to linear and a logarithmic regression equation obtained by computer analysis f the test material These were superior to all other methods. The logarithmic regression equation was slightly more accurate than the linear equation, but the differences was not significant. Simonson et al. found a small but significant increase in Q-T duration with age and incorporated an age correction

Q-T = 242.3 + 0 140 R R + 0.3 age (Q-T in msec.)

The standard error f estimate was 16.4 msec. The formula is valid for heart rates between 45 and 115 which may explain the discrepancy with the formulas derived from data in this study The slope of the regression equations for Groups OC and YC would have been less steep if the ECGs at rest had been included in the analysis The Q-T duration difference f 9 msec. between Group OC and YC at similar heart rates at rest is equal to the d fference predicted by Simonson s age factor

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Linear pattern class fication The LCTP was applied to data i this series mainly to investigate whether the procedure could be used as means of evaluating the relative

mportance of each variable in comparisons etween groups characterized by large num ers of measurements. The results agreed vith the results of conventional item ana ysis

More detailed studies involving also other nethods of pattern classification should be carried out to support the validity of the method in this application but the results suggest that ECTP and related procedures may represent useful methods to screen large number of variables and delineate complex group differences.

CHAPTER VII

ELECTROCARDIOGRAPHIC CHANGES DURING AND AFTER EXERCISE IN PATIENTS WITH ANGINA PECTORIS EVALUATED BY THE ERROR CORRECTING TRAINING PROCEDURE SUPPLEMENTED WITH NUMERICAL ANALYSIS METHODS

The error-correcting training procedure (ECTP) has been described in Chapter V Two aspects f the method have been con sidered in the present study the use of the results of the classification procedure as a quantitative diagnostic index, and the infor mation content of the weight factors included in the linear discriminant function generated by the ECTP The weight factors give a quantitative evaluation of the signif cance of each variable provided the validity of the discriminant function can be established

A numerical analysis of single ECG items has been performed to supplement data derived from the ECTP and to provide basis for an evaluation of the performance of the ECTP

Material

Control gro p Groups OC and YC (Chapter VI) served as control groups.

A gina patient Group AP The following criteria were used to select male angina pectoris patients for the study typical angina on effort, regularly provoked at a relatively fixed level of physical activity the pain being substernal with or without radiation and disappearing within ten minutes upon termination of exercise, no clinical evidence of past myocardial inf rction, blood pressure of 160/95 or below no diabetes an essentially normal heart olume and resting ECG and age range corresponding to that of Group OC.

A total of 10 patients were collected from the files of the Departments of Medicine and Clinical Physiology Serafimerlasarettet. Three of these fell outside the age range of the control group OC. They were 43 58 and 63 years old. The mean age of Group AP was 51.7 ± 1.8 S.D 6.1 years, which did not differ significantly from the mean ge of group OC, 52.1 years.

All members of Group AP had been studied at routine clinical exercise tests prior to the present investigation. All had also showed changes in conventional ECG leads which had been interpreted as being due to ischemia.

Patients in Group AP had had angina for an a erage of 2.1 years, range 3 months to 6 years, with no change in severity of symptoms during the month immediately preceding the study Coronary angiography was performed in 5 cases One man had complete occlusion of two major branches, two men of one major branch. No occlusions but definite coronary artery changes were present in two patients.

The mean relative heart olume (standing position, ml./B.S.A.) was 430 ± 25 ml., S.D 83 which did not differ significantly from the mean volume in Group OC, 420

ml Three patients had heart volumes exceeding 500 ml/B.S.A with values of 530 530 and 540

There were no significant differences in blood pressure between the two groups Mean systolic blood pressure was 144 0 ± 1 7 S D 5 3 mm Hg in Group AP versus 138 8 in Group OC. Corresponding figures for diastolic pressure were 88 5 ± 1 6 S D 5 3 mm Hg, versus 86 0

The men in Group OC were taller and heavier with a B.S A of 1 95 versus 1 81 ± 0 05 S D 0 14 m² in Group AP

All patients in Group AP were occupationally active at the time of the examination. No patient was on digitalis or had been taking any diuretic nitrite or other drug smoked or had any meal at such intervals prior to the study as were likely to influence the results of the exercise test (Simonson 1961)

Methods

A mechanically braked bicycle ergometer (Monark) was used in all experiments in Group AP Other methods conformed to those described in Chapter VI

Procedure

The procedure in Groups OC and YC has been described in Chapter VI Five of the ten patients in Group AP were studied 3 times during an 8-week periods and a single experiment was carried out in five men.

Work loads were selected according to the results of previous exercise tests to include at least one load below the threshold of anginal pain and (except in one patient) one load sufficent to provoke angina. The sub-threshold level was 300 kpm/min. in 7 patients, and 150 200 and 450 in the three remaining men The mean load was 290 kpm/min., and it was considered equal to 300 kpm/min. in the analyses. The threshold level was 600 kpm/min in 7 patients, 450 in one, and 900 in one patient The mean load was 617 kpm/min., and it has been referred to as 600 kpm/min.

The exercise test was preceeded by a rest period of 20 to 30 minutes. A complete 12 lead conventional ECG was recorded The patient then transferred to a chair for a resting Frank lead ECG Frank leads were also recorded on magnetic tape during the last minute of exercise, and immediately after and three minutes after exercise CH leads wer recorded immediately after Frank leads during work and immediately before Frank leads post-exercise. The ECG was monitored on an oscilloscope. A rest period of 15 to 30 minutes was inserted between the two loads The second part of the exercise test was not started until heart rate and scalar ECG amplitudes had returned to the level before exercise. The duration of work was 6 minutes at the sub-threshold level Exercise at the higher load was terminated within 30 seconds after onset of any anginal pain. Anginal pain was the only reason for discontinuing exercise before a standard six minute period was completed Arrhythmias other than occasional premature beats were not observed

Results

Symptoms No patient experienced any chest discomfort at the 300 kpm/min. level. All patients developed light or moderately severe angina after 3 to 5 minutes exercise at the 600 kpm/min level The pain disappeared within 30 seconds postexercise in most patients and did not last for more than 2 minutes in any case

Intraindividual variability with regard to the duration of exercise required to provoke angina pectoris at a given external load was studied in the subgroup of 5 patients examined 3 times. The time of onset of angina remained stable within ± 1/2 minute.

Heart rates during exercise. The mean heart rate during exercise at 300 kpm/min. was 100.1 ± 3.8, S.D. 12.6, which did not differ significantly from the mean value in Group OC, 94.8. The heart rate during 600 kpm/min. in Group AP 131.4 ± 6.6, S.D. 16.8 which did not differ significantly from the heart rate in Group OC at relative load of 60 per cent, 133.1.

Lactate. The mean arterial lactate in 5 patients in Group AP at 600 kpm/min. was 26.0 mg per cent. Corresponding value in Group OC at the 60 per relative load was 31.4. The difference was not significant.

Electrocardiographic findings

Only results from comparisons between Groups AP and OC will be reported. Differences between Groups OC and YC were described in Chapter VI.

ECG data are presented in Fig. 12 (Group OC) and 13 (Group YC) Chapter VI, Fig. 17 (Group AP) and Fig. 18 (Group AP and OC).

Numerical analysis of amplitudes

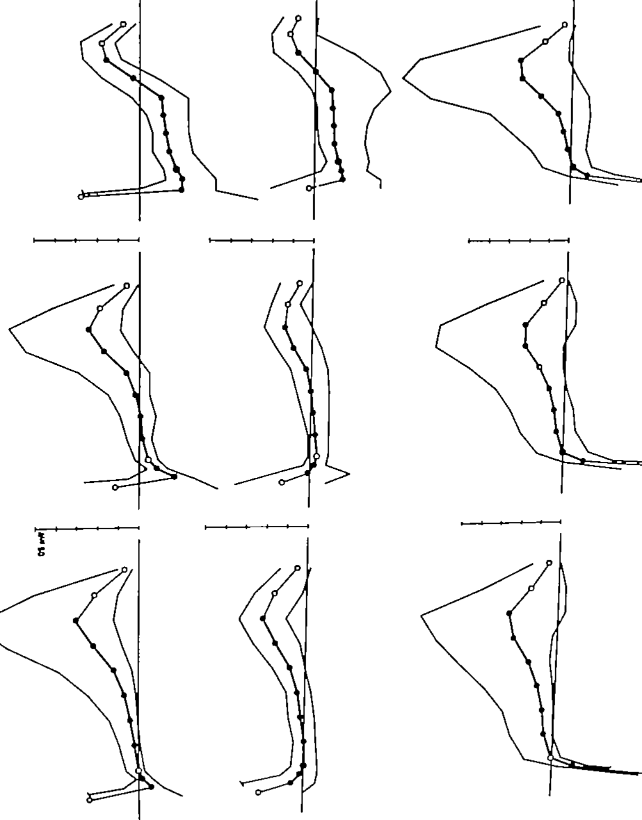
Conventional and Frank ECG at rest. Conventional 12 lead ECGs at rest were analyzed according to the Minnesota code (Blackburn et al. 1960). There were only two items fulfilling any criteria of the code. One man had tall, peaked T waves in leads V_4 and V_5 with maximal amplitude of 1.5 mV and one man had a diphasic T wave in leads V_1 and V_2. The amplitude of the terminal negative phase was less than 0.1 mV.

The mean values of the Frank lead 8/8 ST-T vector components closely corresponded to those given by Draper et al. (1964) but ranges were exceeded in both directions. The upper range of QRS vectors 3, 4, and 5 was also exceeded by one patient. Fig. 18. A demonstrates that there were only minor differences between Groups OC and AP regard to X and Y ST-T components at rest. The two cases with reportable T wave findings according to the Minnesota code in the conventional 12 lead ECG at rest also defined upper and lower ranges in the Z lead.

CH leads at 300 kpm/min. Two of the ten patients in Group AP did not show any ST-T abnormalities at the sub-threshold level of 300 kpm/min. The most common pattern, exhibited by 4 patients, was a J depression of 0.05 to 0.10 mV and a straight but ascending ST segment and no T wave abnormalities during and immediately after work, and no ST-T changes 3 minutes after work. Similar ST changes were present during, immediately after and three minutes after work in one patient. Two patients had a strictly horizontal depression of 0.05 mV during work. One of these showed a horizontal depression of 0.20 mV 3 minutes after work. One patient, finally had a strictly horizontal depression of 0.10 mV during and immediately after work but no changes 3 minutes after work.

There were no ST-T changes in Group OC at 300 kpm/min.

CH leads at 600 kpm/min. — 60 per cent relative load. Eight of the nine patients in Group AP studied at 600 kpm/min. had a strictly horizontal ST depression of 0.10 mV



or more in one or more of the CH leads during and/or after exercise

Seven patients demonstrated horizontal depression during work (range 0.10 to 0.60 mV) as well as immediately after (0.10 to 0.40 mV) and 3 minutes after work (0.05 to 0.30 mV). Five of these patients had inverted or diphasic T waves 3 minutes after exercise. One patient had a horizontal depression of 0.20 mV during and immediately after work decreasing to 0.05 mV 3 minutes after exercise. One patient did not show any strictly horizontal depression at any time at this load. He had a J depression of 0.15 mV during work with a straight but ascending ST segment and no ST T changes 3 minutes after work. The appearance of the ST segment in lead CH_6 during work closely corresponded to that of the upper range in the X lead. Fig. 17 c.

Thus 8 of 9 patients in Group AP developed definite ST changes of the ischemic type at 600 kpm/min. No subject in Group OC showed any horizontal ST depression in CH leads at the 60 per cent level.

ECG changes in Frank lead during and after exercise. Frank leads at rest and during exercise at 300 kpm/min, and at the 60 per cent level, i.e. mean X, Y and Z amplitudes and range for ST T vector components in Groups OC and YC appear in Fig. 12 and 13 Chapter VI. Mean values and ranges for terminal QRS and ST T vector components in Group AP at rest and during exercise at 300 and 600 kpm/min. are shown in Fig. 17. Fig. 18 compares QRS 6, 7 and 8 and ST T 1 through 6 in Groups AP and OC at rest and during exercise 60 per cent at 600 kpm/min.

300 kpm/min. Numerical comparisons between Groups OC and AP at the 300 kpm/min. level revealed probably significant or significant differences only in the Y lead for ST T component 2 to 6. Differences were smaller and not significant in recordings after exercise.

600 kpm/min — 60 per cent relative load. Fig. 18 shows that there was a complete separation of Groups AP and OC with regard to X components of ST T vectors 2 and 3 during work. Intergroup differences were highly significant in the X lead for all components QRS 8 to ST T 5 with highest t-values for ST T 2 and 3. Y lead differences with regard to QRS 8 and ST T 1 through 4 were also highly significant and ST T 5 and 6 significant, but t values were generally somewhat lower than for corresponding components in the X lead. There was a probably significant difference in QRS 8 or J amplitudes in the Z lead, but other differences were not significant.

Fig. 1. Group mean in normal QRS and ST T vector components and ST T range in patients with angina pectoris at rest and during exercise at 300 and 600 kpm/min. (Group AP)

Top lead X, middle Y, bottom Z.

Filled circles denote vector components included in the ECTP analysis.

( ) Rest, heart rate 70, N = 10

(b) During exercise at 300 kpm/min., heart rate 100, N = 10. No angina in any patient.

( ) During exercise at 600 kpm/min., heart rate 131, N = 9. Definite angina in all patients.

5

Quantitative evaluation of changes in series of ECGs at rest and during after exercise by means of the error correcting training procedure

Preliminary studies involving inter and intragroup comparisons of data including

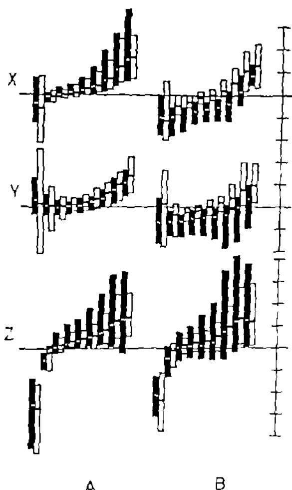


Fig 18 Gro p m nd ng f terminal QRS and ST T ector ampe u re per cent u th ang na pectors (G oup AP) and middl -aged c ntrols (Groups OC) at u nd dur g exerc e

Each pair of filled (Group AP) and open (Group OC) bars represents range and mean amplitude for a vector component (QRS 6, 7 and 8, ST T 1 through 6)

(A) Rest, (B) During exercise at 600 kpm/min. — 60 per cent relati e load

P 0 and P QRS, and ST T 8/8 X, Y and Z vector components in Groups AP and OC, i.e. sets of 75 amplitude measurements from each ECG indicated that the largest intergroup differences were to be found in the terminal QRS vectors and during the first 3/4 of the ST T segment as judged from the *per cent contribution* of each variable calculated from the magnitude of the weight factors. Accordingly all comparisons in this chapter have been based on sets of amplitude measurements consisting of X, Y and Z components of QRS vectors 6, 7 and 8, and ST T vectors 1 through 6. ECGs recorded at rest, during exercise, and imme diately after and three minutes after exercise at 300 or 600 kpm/min. in Group AP and at corresponding loads in the control groups OC or YC have been analyzed simulta neously Comparisons thus included from *each individual 27 amplitude measurements* from 4 ECGs or a total of 108.

Groups AP and OC at 600 kpm/min — 60 per cent relative load

The results of a comparison between Group AP and OC at the 600 kpm/min. — 60 per cent level were studied in detail

A separating hyperplane was found with a safety margin, r of ± 34 units The mean *distance to the hyperplane, or the score,* was + 43 units in Group AP and —76 units in Group OC.

Results of the classification p ocedure Fig 19 summarizes the results of applica *tion of the discriminant function defined* by Groups AP and OC at the 600 kpm/min. — 60 per cent level The top row (a) shows the classification of individual ECG series in these two groups.

b) includes 5 of the cases in Group AP each patient studied on two additional occa

sions. A mean decrease in distance to the separating plane by 29 units (probably significant) was found for the ECGs from the first of these studies, and by 26 units (not significant) for those from the second study. In each comparison only two of the five men showed differences compared to the records included among those defining the hyperplane. The decrease in distance to the separating hyperplane was in each case parallelled by decrease in ST depression in CH leads.

Row (c) shows the results of an evaluation of ECGs in Group AP at the sub-threshold level, 300 kpm/min. The two patients who demonstrated horizontal ST depression exceeding 0.1 mV were classified accordingly.

Row (d) includes angina patients studied during arm work and work in cold environment (Chapter VIII). No CH leads were recorded in the patient represented by two filled squares on the left side. A definite ST depression was present only in the Y lead. This man also had signs of emphysema.

Row ( ) shows the results of the evaluation of two sets of ECGs recorded at 600 kpm/min in Group OC. The mean score was —67 units at the first examination, and —71 units at the second. The difference was not significant. The standard error of the method (including biological variability) calculated from these data was ± 7 per cent.

No subject in Group OC showed significant horizontal ST depression in CH or Frank leads (Fig. 18 indicates the range) at the 60 per cent level, but the six subjects who developed horizontal ST depression in CH leads at maximal loads were significantly closer to the separating hyperplane than the rest of the group with mean score of —45

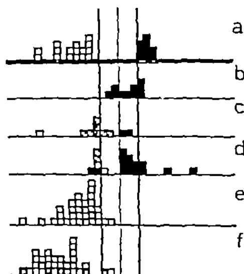


Fig. 19. Results of application of discriminant function generated by ECTP from ECG data at rest, during, immediately after and 3 minutes after exercise at 600 kpm/min — 60 per cent in patients with angina pectoris (Group AP) and middle-aged control (Group OC).

Open squares denote control subjects, cross-hatched control subjects during acute hypoxia.

Filled squares represent patients with angina pectoris and definite ischemic ST depression in scalar leads, open squares with oblique bars patients with angina pectoris but with only minor, qualitative ST-T changes, and open squares with dots angina patients with normal ECG.

For further explanation, see text.

units compared to —69 units for the group as a whole.

Row (f) displays the results in Group YC and in the young normal subjects studied during acute hypoxia (Chapter IX). The mean distance to the separating hyperplane was significantly larger for Group YC than for OC, —101 units versus —69.

Weight factor. The weight factors defining the hyperplane separating Groups AP and OC at the 600—60 per cent level are

Table I Weight factors of a linear discriminan function generated by the error-correcting procedure

Comparison of Group AP and OC at 600 kpm/min. — 60 percent relative load. C = *per cent contribution of each QRS and ST T* vector

		X	Y	Z	C		X	Y	Z	C		X	Y	Z	C
Rest	QRS 6	—6	14	— 3	7 6	QRS 7	—1	8	—13	2.3	QRS 8	1	—1	—4	0
Ex		—3	—11	5	1 6		—14	—1	0	3 4		—19	—14	—4	5 7
Imm.		21	—5	4	4 8		—6	—2	—18	3 6		—7	—3	—9	1 4
3 min.		14	7	1	2 5		1	2	5	0 1		—2	—3	3	0.2
Rest	ST T 1	1	—4	—3	0.3	ST T 2	4	—3	—2	0.3	T T 3	3	—5	—4	0.5
Ex		—18	—15	—4	5 7		—19	—17	—5	6.8		—19	—19	—5	7.3
Imm.		—5	—4	—10	1 4		—8	—7	—11	2 5		—9	—8	—13	3 1
3 min.		—4	—5	3	0 4		—2	—6	1	0 4		—6	—7	—	0 9
Rest	ST T 4	4	—5	—	0 5	ST T 5	5	—7	1	0 8	T T 6	5	—7	5	1.0
Ex		—18	—19	—4	7 1		—13	—12		3.2		0	—5	5	0 5
Imm.		—15	—10	—19	3.6		—9	—6	—15	3 4		6	6	—11	1.9
3 min.		—9	—10	—5	2.1		—16	—13	—9	5 1		—16	—16	—5	5 4

listed in Table I The table also lists the percentage of the total difference between the two groups contributed by each QRS and ST T vector which is equal to the sum of the squared X, Y and Z weight factors divided by 100

There was generally a good agreement between the magnitude of individual weight factors and the results of the statistical analysis of amplitude data reported above

The highest weight factors for ST T components during work in the comparisons between Group OC and AP were found for ST T 2 and 3 in the X and the Y lead (Cf Fig 18) However the agreement between the results of t-tests and weight factors was not absolute, e g t-test showed a significant intergroup amplitude difference during exercise in the X component of ST T 6, but the corresponding weight factor was zero indicating that the difference did not contribute to the separation by the linear discriminant function

The percentages derived from the weight factors measuring contribution of each vector component indicated that 13 4 per cent of the total difference between the two groups was due to differences in the ECG at rest, as compared to 41 per cent during exercise, 30 per cent immediately after and 17 per cent 3 minutes after exercise.

The large weight factors were distributed differently on the various subsegments of the ST T interval in the series of four ECGs included in the analysis. ST T 2, 3 and 4 were most important during exercise ST T 3 4, and 5 immediately after exercise, and ST T 5 and 6 3 minutes after work.

The contribution of each lead also varied with the time of recording Twenty-three per cent of the total intergroup difference was attributed to the X lead during work

Corresponding figures for the Y and Z lead were 19 and 2 per cent. The Z lead was the relatively most important lead immediately after work with a total contribution of 15 per cent versus 11 per cent for lead X and 4 for lead Y. Percentages 3 minutes after exercise were 9 for lead X, 7 for Y and 1 for Z. The total contribution for the entire ECG series was 43 per cent for X, 33 for Y and 24 for Z.

Comparisons Involving Other Groups and Work Load Level

Groups AP and OC at 300 kpm/min. A separating hyperplane was found, but the safety margin between the two groups was smaller than at the higher work load level, ± 3 units. Fig. 17 b also shows that there were patients in Group AP with no ST depression during exercise at 300 kpm/min. However the discriminant function classified correctly all ECG series from repeat studies in 3 patients in Group AC. All ECG series from the angina patients described in Chapter VIII except 4 of total of 22 were classified as belonging to the angina group. Scalar leads did not reveal any abnormalities in these 4 cases. All subjects in Group YC were classified as normal. The distribution of weight factors differed in some respects from that at the 600—60 per cent level. The proportion of the total difference contributed by the ECG during exercise was the same, or 41 per cent, but 27 per cent was attributed to differences in the ECGs at rest, and 11 per cent to the ECG immediately after and 21 per cent to the ECG 3 minutes after exercise. ST T vector 4 during work was the most important single item in the entire series and defined 9 per cent of the total difference. The total contribution of ST T 4 was also the highest, 15 per cent of the total difference as compared to e.g. 4.5 per cent for QRS 8 (J), 9 per cent for ST T 3, 12.4 per cent for ST T 5 and 12 per cent for ST T 6 (maximal T).

Groups YC and AP. Discriminant functions defined by comparisons of Groups AP and YC generally provided a wide separation of the groups. *Test runs classified a* large proportion of the subjects in Group OC among the angina patients at the 300 kpm/min. level. ST T 4 was the most important of all subsegments according to the weight factors.

The discriminant function derived from comparisons of Groups AP and YC at the 600—60 per cent level classified all subjects in Group OC correctly. The results in angina patients were generally in agreement with those reported above for the function derived from Groups AP and OC. The distribution of weight factors was also similar with 42 per cent of the total difference attributed to *changes during exercise. Corresponding* percentages for immediately after and 3 minutes after exercise were 27 and 18 per cent. ST T 4 was also in this comparison the most important of all subsegments.

Discussion

Material

Group AP is obviously representativ only of small fraction of all patients with coronary heart disease. The group was selected to provide laboratory group of patients with definite clinical evidence of coronary heart disease demonstrating a minimal amount of abnormalities in other respects. The investigation a character of pilot study is also underlined by the small number of subjects in the control groups and by the fact that no patients with circulatory disorders not primarily affecting the coro-

Table I Weight factors of a linear discriminan function generated by the error-correcting procedure.

Comparison of Group AP and OC at 600 kpm/min — 60 percent relative load. C = per cent contribution of each QRS and ST T vector

		X	Y	Z	C		X	Y	Z	C		X	Y	Z	C
Rest	QRS 6	—6	14	—23	7 6	QRS 7	—1	8	—15	‿3	QRS 8	1	—1	—4	0 2
Ex.		—3	—11	5	1 6		—14	—12	0	3 4		—19	—14	—4	5 7
Imm.		21	—5	4	4.8		—6	—2	—18	3.6		—7	—3	—9	1 4
3 min		14	7	1	2.5		1	2	3	0 1		—2	—3	3	0 2
Rest	ST T 1	1	—4	—3	0.3	ST T	4	—3	—2	0.3	T T 3	3	—5	—4	0.5
Ex.		—18	—15	—4	5 7		—19	—17	—5	6 8		—19	—19	—5	7.5
Imm		—5	—4	—10	1 4		—8	—7	—11	2.3		—9	—8	—13	3 1
3 min.		—3	—5	3	0 4		—2	—6	1	0 4		—6	—7	—2	0 9
Rest	ST T 4	4	—5	—2	0 5	ST T 5	5	—7	1	0.8	T T 6	5	—7	5	1.0
E		—18	—19	—1	7 1		—13	—1	2	3.2		0	—5	5	0.5
Imm.		—15	—10	—19	3.6		—9	—6	—15	3 4		6	6	—11	1.9
3 min.		—9	—10	—5	2 1		—16	—13	—9	5 1		—16	—16	—5	5 4

listed in Table I The table also lists the percentage of the total difference between the two groups contributed by each QRS and ST T vector which is equal to the sum of the squared X Y and Z weight factors divided by 100

There was generally a good agreement between the magnitude of individual weight factors and the results of the statistical analysis of amplitude data reported above

The highest weight factors for ST T components during work in the comparisons between Group OC and AP were found for ST T 2 and 3 in the X and the Y lead (Cf Fig 18) However the agreement between the results of *t* tests and weight factors was not absolute e.g *t* test showed a significant intergroup amplitude difference during exercise in the X component of ST T 6, but the corresponding weight factor was zero indicating that the difference did not contribute to the separation by the linear discriminant function

The percentages derived from the weight factors measuring contribution of each vector component indicated that 13 4 per cent of the total difference between the two groups was due to differences in the ECG at rest, as compared to 41 per cent during exercise, 30 per cent immediately after and 17 per cent 3 minutes after exercise.

The large weight factors were distributed differently on the various subsegments of the ST T interval in the series of four ECGs included in the analysis. ST T 2, 3 and 4 were most important during exercise, ST T 3 4, and 5 immediately after exercise and ST T 5 and 6 3 minutes after work.

The contribution of each lead also varied with the time of recording. Twenty-three per cent of the total intergroup difference was attributed to the X lead during work.

tion of the two groups. Amplitude data at this level thus primarily provided material for an evaluation of quantitative results of the classification process.

Results of the ECTP The results of the classification procedure based on the discriminant function defined by Groups AP and OC at the 600 kpm/min. — 60 per cent level appear to establish the validity of the discriminant function and thereby also the validity of the information contained in the weight factors. There was a relatively good agreement between the results of the t-tests of amplitude differences in separate orthogonal leads and the weight factors. The distribution of the weight factors (Table 1) demonstrates that most of the total group difference during exercise is attributed to a horizontal ST depression in leads X and Y Negative amplitude values in X and Y at ST T 1 through 5 will classify an ECG series belonging to the angina group.

The results in the test groups also validated the discriminant function defined by Groups AP and OC at 300 kpm/min. despite the large amplitude overlap Important weight factors were concentrated to amplitudes corresponding to the second half of the ST segment and the first half f the T wave

The results from the comparisons between Groups AP and YC largely corresponded to those discussed above egard to the weight vectors.

A comprehensive analysis of the weight factors suggested that a lead closely corresponding to CH 5 would theoretically contain maximal amount of information. The conclusion was based on calculations of the per cent contribution of each lead and data on lead characteristics derived from studies on torso models (Blackburn and Rautaharju, unpublished observations) Furthermore an analysis limited to a single amplitude measurement should preferably employ ST T 4, i.e. the amplitude at half the distance between the end of the QRS complex and the end of the T wave.

The significance of the shape of the ST segment was established primarily by the follow-up studies reported by Robb Marks, and Mattingly (1957) and Mattingly (1962) Data on the rarity of significant horizontal ST depression in young healthy men and on the increasing frequency in higher age groups in men with no clinical evidence of coronary heart disease have recently been reviewed by Doan et al (1965) Extensive review have also been published by Sherf and Schaffer (1952) Lepeschkin and Surawicz (1958) and Bellet (1965)

In summary a diagnostic procedure that generates its own criteria during the analysis process defined criteria closely corresponding to measurement of the degree of horizontal ST depression, or the degree of ST depression to a duration corresponding to approximately 50 per cent of the interval between the end f the QRS and the end of the T wave

The results suggest that the ECTP in some diagnostic applications may provide an alternative to methods based on probability densities (Pipberger and Stallman 1964, Arvedson 1965) which require relatively large basal materials.

nary arteries have been studied to evaluate the specificity of the results. All patients in the test group had furthermore demonstrated *significant* ECG changes during exercise according to standard methods of interpretation.

Procedure

The angina group was relatively homogeneous with regard to the work load level required to provoke anginal pain. The work load level in Group OC selected for comparisons with Group AP at 600 kpm/min. was 60 per cent the relative load rather than the identical external load, *primarily* because of the closer agreement with regard to heart rate during exercise and the smaller intragroup variability among the controls.

Symptoms

The findings in the subgroup of 5 angina patients studied on three occasions support the observation by Areskog and Hallén (1964) *that the time of onset of angina* during work shows a high degree of reproducibility at repeated exercise tests.

Electrocardiographic findings

The results of conventional analysis of findings in CH leads and numerical analysis of the amplitudes *in Frank leads were primarily* reported to provide a basis for an evaluation of the performance of the ECTP.

It should be recalled from the presentation of the ECTP (Chapter V) that the procedure does not allow any errors. This may lead to the definition of a discriminant function that represents a distorted picture of the group differences. Some information on the degree of distortion could probably have been gained by comparison of the results of the ECTP with the results of other pattern-classifying methods. Empirical validation of the discriminant functions was however considered sufficient for the purposes of this pilot study, i.e. the discriminant function has been validated by *observing the results of the* classification procedure in various test groups and relating them to results obtained by other methods.

CH and Frank leads at 300 kpm/min. Analysis of CH and Frank leads in Group AP during exercise at 300 kpm/min. demonstrated a *large intragroup variability with* responses ranging from a horizontal ST depression exceeding 0.10 mV to no appreciable ECG changes. Statistical evaluation of differences between mean values in Groups AP and OC showed significant ST-T differences in the Y lead, but ranges were overlapping *to a great extent. Amplitude measurements* at this level were thus likely to provide an interesting test material for an evaluation of the diagnostic efficiency of the ECTP.

CH and Frank leads at 600 kpm/min. — 60 per cent relative load. The results of the analysis of changes in CH leads and the numerical analysis of amplitude data in the Frank leads at the 600 kpm/min. — 60 per cent level stress the fact that Group AP should be regarded as a highly selected test group. CH leads showed definite ischemic *changes according to all criteria (see a review* by Simonson 1963) in all patients but one who had a nearly horizontal ST depression and fulfilled e.g. Master's revised and Lepeschkin's and Surawicz's criteria of an ischemic *ST* depression. *Fig.* 18 b also demonstrates that measurement of single amplitude items (X components of ST-T 2 and 3 during work) sufficed to separate the two groups. This separation also ensured that the ECTP would effect a complete separa-

blood pressure 170/100 mm Hg. He also had a relative heart volume (standing position, ml./B.S.A.) at the upper normal limit, 530 ml. The mean value for the group was 445 ± 30 ml.

Arm Work Group AA. The mean age of the six men in this group was 57.0 years (range 46 to 49). One man had been hospitalized for a myocardial infarction 6 years ago. All had had angina for more than 2 years (mean 3.8 range 2 to 6 years). Coronary angiography in 5 patients demonstrated definite coronary artery changes in 5 and occlusion of one major branch in 3 patients. Mean systolic blood pressure was 157.5 ± 7.2, and mean diastolic 97.5 ± 4.8 mm Hg. Only one patient had pressures below 150/100. Mean relative heart volume was 400 ± 50 ml.

One patient had laboratory evidence of a moderate degree of pulmonary emphysema.

Methods

ECG method have been described in Section I. Arm and leg work was performed in sitting position on a mechanically braked *bicycle ergometer* (Monark) at pedal frequency of 50 rev/min. *Oxygen uptake* was determined by collection of expired air in Douglas bags and analysis in modified Haldane apparatus. During arm work the meter was placed on a stand in front of the patient with the hub on shoulder level. The pedals were replaced with special handles. Only *indirect blood pressure* were recorded in Group AC. *Intraarterial pressure* were obtained in Group AA. A teflon catheter was inserted percutaneously into the brachial artery. The total length of the catheter was 60 cm., and the intravascular portion approximately 15 cm. An Elema-Schönander transducer and electromanometer were used.

Heart rates were determined from the ECG as an average of at least 12 R-R intervals.

Symptoms The patients were asked to grade their symptoms of angina and dyspnoe at successive loads simply as "more", "less" and "no change". The duration of work required to precipitate angina was recorded.

Procedure

The work loads at the control study (leg exercise at normal room temperature in both groups) were based on the results at previous exercise tests and selected to provoke angina of light intensity at the end of a six minute period of exercise. The exercise was interrupted upon the appearance of definite symptoms of angina. The ECG was monitored on an oscilloscope.

ECG and blood pressures were recorded (sitting position) after an initial rest period of 20 to 30 minutes. Pulse rates and blood pressures were recorded at 2 mm. intervals during work. Collection of expiratory air was started as soon as the patient experienced any angina, or at the beginning of the sixth minute of exercise. A tape recording of the ECG during exercise was made simultaneously. ECG recording were repeated immediately after, and 3 minutes after exercise.

The patient rested for a period varying between 15 and 45 minutes until the resting scalar ECG had returned to its appearance before exercise. The exercise procedure was then repeated starting with ECG and blood pressure recordings at rest.

Work in cold environment The control study at normal room temperature (range 18 to 23 centigrades) was carried out first in all experiments. The work load varied between 200 and 600 kpm/min., mean 400.

The patient and the bicycle ergometer

CLINICAL, PHYSIOLOGICAL, AND ELECTROCARDIOGRAPHIC FINDINGS IN PATIENTS WITH ANGINA PECTORIS DURING WORK IN COLD ENVIRONMENT AND DURING ARM WORK

by

G Blomqvist Irma Åstrand and *R Messin*[1]

Patients with angina pectoris commonly experience a decreased exercise tolerance during cold weather Chilling of the nose or the hands or ingestion of cold drinks, may also precipitate angina. These changes have been attributed to coronary vasomotor reflexes rather than to an increased cardiac work (Freedberg et al 1944)

It is also a clinical experience that cardiac patients coronary and non-coronary who accurately know their tolerance limits for walking, may overstrain themselves if they occasionally engage in activities predominantly requiring arm work. Studies in normal subjects have demonstrated that heart rate and peripheral resistance are higher during arm work than during leg work at loads demanding equal oxygen uptake (Åstrand et al. 1965)

These observations suggested two experimental procedures to modify the relationship between myocardial oxygen demand and supply while keeping the work load constant in terms of total body oxygen uptake Two small groups of patients with angina pectoris were studied one during work in cold environment and at normal room temperature, and one during arm and leg work.

Material

All patients included in the study presented a typical history of angina on effort. Angina pectoris and ECG changes compatible with coronary disease had been provoked at routine exercise tests prior to the present study No changes in severity or frequency of attacks had occured in any patient during the last few months. No patient was on digitalis therapy

Work in cold environment Group AC The group included five male patients with a mean age of 58 0 years (range 47 to 63) One man had evidence (ECG and history) of a healed myocardial infarction All had had attacks of angina for one year or more (mean 3 4 range 1 to 6 years)

Coronary angiography had been performed in three patients. All showed definite coronary artery disease Two had occlusion of two major branches. No occlusions could be demonstrated in the third patient.

Mean systolic and diastolic blood pressures at rest were 138.0 ± 9 7 and 90.0 ± 7 1 mm Hg Only one patient had an elevated

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blood pressure, 170/100 mm Hg. He also had a relative heart volume (standing position, ml./B.S.A.) at the upper normal limit, 530 ml. The mean value for the group was 445 ± 30 ml.

Arm Work Group AA The mean age of the six men in this group was 57.0 years (range 46 to 49) One man had been hospitalized for a myocardial infarction 6 years go. All had had angina for more than 2 years (mean 3.8 range 2 to 6 years) Coronary angiography in 5 patients demonstrated definite coronary artery changes in 5 and occlusion of one major branch in 3 patients. Mean systolic blood pressure was 157.5 ± 7.2, and mean diastolic 97 5 ± 4.8 mm Hg. Only one patient had pressures below 150/100. Mean relative heart olume was 400 ± 50 ml.

One patient had laboratory evidence of a moderate degree of pulmonary emphysema.

Methods

ECG methods have been described in Section I Arm and leg work was performed in sitting position on mechanically braked *bicycl ergometer* (Monark) at a pedal frequency of 50 rev/min. *Oxyg uptak* was determined by collection of expired air in Douglas bags and analysis in a modified Haldane apparatus. During arm work the meter was placed on stand in front of the patient with the hub on shoulder level. The pedals were replaced with special handles. Only *indirect blood pressure* were recorded in Group AC *Int aarterial pre sure* were obtained in Group AA A teflon catheter was inserted percutaneously into the brachial artery The total length of the catheter was 60 cm and the intravascular portion approximately 15 cm. An Elema Schönander transducer and electromanometer were used.

Heart rates were determined from the ECG as an average of at least 12 R-R intervals.

Sympt m The patients were asked to grade their symptoms of angina and dyspnoe at successive loads simply as more "less and no change The duration of work required to precipitate angina was recorded.

Procedure

The work loads at the control study (leg exercise at normal room temperature in both groups) were based on the results at previous exercise tests and selected to provoke angina of light intensity at the end of a six minute period of exercise The exercise was interrupted upon the appearance of definite symptoms of angina. The ECG was monitored on an oscilloscope.

ECG and blood pressures were recorded (sitting position) after an initial rest period of 20 to 30 minutes Pulse rates and blood pressures were recorded at 2 min. intervals during work. Collection of expiratory air was started as soon as the patient experienced any angina, or at the beginning of the sixth minute of exercise. A tape recording of the ECG during exercise was made simultaneously ECG recording were repeated immediately after, and 3 minutes after exercise.

The patient rested for a period varying between 15 and 45 minutes until the resting scalar ECG had returned to its appearance before exercise. The exercise procedure was then repeated starting with ECG and blood pressure recordings at rest.

Work in cold environment The control study at normal room temperature (range 18 to 23 centigrades) was carried out *first* in all experiments. The work load varied between 200 and 600 kpm/min., mean 400

The patient and the bicycle ergometer

Table I

Work in cold environment   Physiological data

n = 5		V_{O_2} lit./min	Ventilation lit./min	Heart rate			Blood pressure during exercise			
				Rest	During exercise		Systolic	Diastolic	Systolic	Diastolic
					2	6'	2'	2'	6	6
Cold	$\bar{x}$ ± S E	1 27±0 14	39.8±5 6	75 4 ± 6.3	116.8 ± 8 4	122.8 ± 11 2	181 0 ± 4 6	102.0 ± 3.0	181.0 ± 4 0	103.0 ± 4 1
	S D	0 32	12.5	14.0	18.8	25 1	10 3	6.7	8 9	9.2
Control	$\bar{x}$ ± S.E	1 18±0 12	34 6±5 7	73 2 ± 5 3	111 4 ± 6.8	113 0 ± 6.8	174.0 ± 8 7	93 0 ± 4 1	181.0 ± 8 1	97.0 ± 3 4
	S D	0 28	12 8	11 9	15 6	15 3	19 5	9.2	18 2	7 7

Table II

Arm and leg work   Physiological data

n = 6		Work load kpm/min	V_{O_2} lit./min.	Heart rate		Blood pressure during exercise			Ventilation
				Rest	During exercise	Systolic	Diastolic	Mean	
Arm work	$\bar{x}$ ± S.E.	215 ± 50	0.87 ± 0 18	71 5 ± 3 1	119.8 ± 8 1	218.2 ± 13 7	126.5 ± 7.6	163.0 ± 8 9	37 2 ± 7 3
	S.D	120	0 43	7 5	20.0	33 5	18.5	21.8	18.0
Leg work	$\bar{x}$ ± S E	340 ± 50	0 94 ± 0 11	71 7 ± 4 1	110.0 ± 9.0	210.8 ± 11 1	114 5 ± 5.6	146 5 ± 6.8	30 7 ± 3 4
	S D	170	0 26	10 0	22.1	27 2	13.8	16.6	8.3

were then transferred to the hospital yard immediately outside the ground level laboratory. Outdoor temperatures at the 5 experiments were + 4 ± 0 —2, —3 and —10 centigrades. No wind-gauge measurements were recorded.

Resting ECG and blood pressures were recorded immediately before the start of exercise. Work loads were equal to those at the control study.

Arm work. The experiment was started with arm work in 2 patients and with leg work in 3. Arm work and leg work had to be carried out on alternate days in one patient in whom the catheter was accidentally pulled out during the last minute of the first experiment.

External work loads during arm work were set to approximately 2/3 of the loads during leg exercise to correct for the lower mechanical efficiency during arm work.

Results

All patients in the two groups according to history and previous exercise tests had relatively sharply defined work load tresholds for anginal pain. This finding was confirmed during the experiments.

Heart rates at rest recorded immediately before the second experiment demonstrated in both groups a return to initial values.

Work in cold environment

Symptoms. One patient did not experience any angina at either study and one patient had angina of equally light intensity during the last minute at both experiments. Two patients had more intense angina at the cold experiment. One man had no angina at the control study but got intense pain after 4 minutes at the cold experiment.

Physiological data. Results are presented in Table I. The oxygen uptake was slightly higher during outdoor work, mean value 1.18 versus 1.27. This trend was consistent in all patients but the difference was not significant. Ventilation was significantly higher during the cold study. Pulse rates and diastolic blood pressure were also higher. The difference in diastolic pressure after 2 min. was probably significant, and the difference at 6 min. was only slightly smaller (p = 0.07). Pulse rate differences were not significant.

The changes demonstrated in Table I were with no exception more marked in the three patients who had more angina during the cold study.

ECG. Group mean X, Y and Z amplitudes were calculated for 8/8 P, QRS and ST-T vectors at rest, and during, immediately after and three minutes after exercise. There were only small and inconsistent differences during P and QRS. Mean X, Y and Z ST-T components during exercise are demonstrated in *Fig. 20.* *Fig. 21* gives mean amplitudes and ranges at rest and during exercise in the cold and control experiment. The amplitude of the horizontal depression in X and Y, i.e. the rightward-upward deviation of the ST vector, was 0.04—0.05 mV larger during the cold experiment, but the difference was not significant.

Individual ECG changes were evaluated by the linear pattern classification procedure (Chapter V), i.e. by comparisons with standard groups, control group OC and the test group of patients with angina at 600 kpm/min. (Chapter VII). QRS 6, 7 and 8 vectors, and ST-T vectors 1 through 6 from the complete series of 4 ECGs from each cold and control experiment defined the individual ECG response. The results were

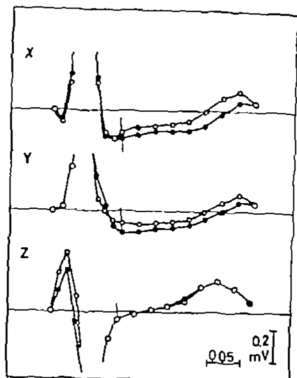
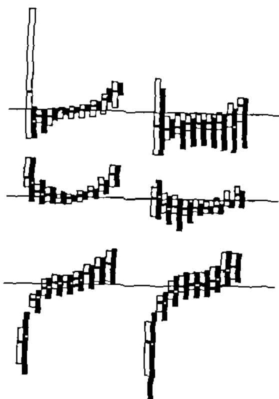


Fig 20 M an ST T mplitudes in Group AC duri g wo k in cold envir ment and at normal oom temperatu

Open circles denote control experiment, filled circles exposure to cold.



F g 1 Group mean nd g of term d QRS and ST T ect omp nent at rest and during work in the cold nd ntrol experim nt in Gro p AC

F lled bars represent ranges and means of ector components QRS 6, and 8, and ST T 1 through 6 t rest (left) and during exercise (right) in the cold experiment, pen bars represent the control study

expressed as the distance to the hyperplane separating the standard groups.

A large positive value indicated that the ECG response markedly differed from that of the control group The group mean distance of the cold study ECG series was 41 and of the preceding control experiment series 26 units The intragroup difference was not significant.

The over all ECG response changed markedly in the abnormal direction in two men at the cold experiment One moved from —50 at the control study to 3 the other from 41 to 103 The first patient had angina of very slight intensity at the control experiment and moderately severe pain at the cold study the second man no angina versus intense pain. These two men also had 15—20 beats higher heart rates during the cold experiment even before any anginal pain had started. Their diastolic blood pressure was consistently 5—10 mm Hg higher Oxygen uptake and ventilation were also higher The third man who had more angina at the cold experiment had less ECG changes as judged from a change from 52 to 31 units. The man who had no angina at either experiment showed a lower pulse rate and no change in blood pressure during the cold study His ECG was less abnormal

with a score of 11 units versus 39 at the control experiment. The fifth patient had moderate angina at both experiments and showed no change in pulse rate or blood pressure. The ECG score was 48 units at the control and 56 units at the cold study

Arm Wo k

Symptoms Both arm work and leg work failed to provoke angina in two patients One patient had no angina during arm work but got moderately severe pain after 4 minutes leg work. Three patients had some angina at both experiments Two men had more intense pain during leg work. Only one man had more severe angina during arm work. Five of the six men in the group had more dyspnoe during arm work.

Physiol gical data Data are presented in Table II There were no significant group differences. The highest t-values were found for diastolic and mean blood pressur (p = 0 06) The mean pressure was 16.5 and the diastolic 12 mm Hg higher during arm work. Systolic blood pressure and heart rate were also slightly higher Ventilation was pproximately 20 per cent highe during arm work Individual differences in oxygen uptake did not exceed 0 10 1/min

ECG The ECG response was evaluated by the same methods as those used for Group AC. Group mean amplitudes are presented in Fig. 22.

ST T vectors 1 to 4 during leg work showed smaller amplitudes in the anterior direction (Z) and an upward displacement (Y) compared to ectors during arm work, while changes were f the same magnitude in the left-to-right direction (X) Analysis of QRS showed that the mean QRS vector (sum of 1 through 8) was more vertical during rm work, and the spatial angle between the mean QRS and mean ST T 1 to 4 vectors was smaller 138 versus 166 degrees. Thus, if the QRS axis had not changed, ST T amplitude differences of approximately equal magnitude probably would have been displayed in all leads.

Comparison of individual data with the standard groups of controls and angina patients by means of the ECTP gave a mean score of —5 units for the control study and of —23 units for arm work. The difference was not significant but indicated that the ECG was less abnormal during arm work.

The single man with more angina during arm work had the same score for both experiments. Only one man had a higher score for arm work. He had no angina at either experiment.

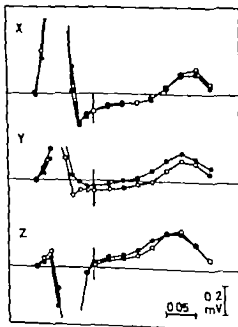


Fig. 22 Mean QRS T amplitude in Gro p AA duri g arm ork and duri g leg ork

Open circles denote arm ork, filled circles leg ork

The remaining 4 patients all had lower scores for arm work. Three of these men had more angina during leg work, and the fourth man no angina at either study

The two men who showed the largest change in abnormal direction with leg work were also the only patients who did not respond to arm work with an increase in mean arterial pressure of 5 mm Hg or more

Comparisons with the standard groups at 300 kpm/min showed corresponding score changes in each case

Discussion

Evaluation of ECG changes in both the cold study and the arm work — leg work experiment by the linear pattern classification procedure and by numerical analysis of amplitudes in individual Frank leads showed a close relationship between the ECG response and the occurrence and severity of anginal pain with definite disagreement only in one patient

Exposure to cold Clinical findings in the cold study were in agreement with earlier observations Freedberg et al. (1944) found a decrease in exercise tolerance and a more abnormal ECG response in a series of 26 patients with angina pectoris with a moderate lowering of the room temperature (from 22 to 24 centigrades to 7 to 13) However local chilling produced identical effects, and there were no changes in heart rate and blood pressure as compared to control experiments at either procedure Freedberg et al attributed the exacerbation of angina to coronary vasomotor changes and pointed out that the results parallelled the experience of many patients that warm clothing leaving only the face exposed cannot prevent a lowering of the treshold of anginal pain during cold weather

The temperature gradient was considerably larger in our experiment. The results, although inconclusive, are compatible with an increased cardiac work and an increased myocardial oxygen demand during the cold experiment. Indirect blood pressure recorded during exercise are open to criticism (Henschel et al 1953) but the workloads were relatively light and the blood pressure measurements thus presented no obvious technical difficulties. The increase in blood pressure was also parallelled by an increase in heart rate during exposure to cold. The three patients who had more angina at the cold study had relatively higher blood pressures and heart rates than the two men who did not get more severe symptoms These differences were present both before and after the onset of angina.

Arm work — leg work Experiments in normal subjects (Åstrand et al. 1965) demonstrated that at equal loads (measured as oxygen uptake) heart rate systolic, diastolic and mean arterial blood pressures, and peripheral resistance were higher during arm work than during leg work. It thus seemed reasonable to postulate that arm work would increase myocardial work and oxygen demand and more easily precipitate angina than leg work of equal intensity but the experimental results of this study did not agree with the hypothesis Heart rate diastolic and mean blood pressure were higher during arm work, but clinical and electrocardiographic findings suggest that myocardial ischemia was more marked during the leg work experiment However it cannot be excluded that myocardial oxygen demands actually were lower during arm work Gorlin et al (1964) have pointed out that any estimate of myocardial energy consumption must include the components of tensile force i.e

intraventricular pressure, intraventricular volume, and the moment-to-moment time course of the two

Furthermore, effects of previous exercise may have introduced bias Findings in this study are compatible with an increased discrepancy between myocardial oxygen supply and demand (as far as can be judged from symptoms of angina nd ECG changes) as an effect of previous exercise The cold study was carried out after the control experiment in all patients. In the arm work — leg work series the second experiment precipitated more angina and/or ECG changes than the first in 4 patients of 5 (2 cases during arm work, and 2 cases during the control study) Data from the literature imply an opposite effect of previous work. Malmborg (1964) has reported lower mean pulmonary artery pressure during a second work period in a series of 9 coronary patients. There was no change in anginal symptoms. W'dimsky et al. (1963) also found a lower mean PA pressure during repeated exercise in 4 normal subjects, and Granath et al. recorded lower pulmonary artery wedge pressures in a series of 9 healthy old men.

More detailed studies of a larger series may reveal interaction between variables explaining the apparent lack of correlation between angina — ECG changes and physiological data in the present study

CHAPTER IX

THE ELECTROCARDIOGRAPHIC RESPONSE TO SUBMAXIMAL AND MAXIMAL WORK DURING ACUTE HYPOXIA

by

G. Blomqvist and J. Stenberg

It has been held (Simonson 1963) that even maximal work loads will not provoke ischemic or segmental ST depressions in young healthy subjects. A survey of the literature also indicates the rarity of horizontal ST depression with exercise in young normal males (Chapter VII).

The present study was undertaken to investigate primarily whether strenuous exercise during hypoxia (at a simulated altitude of 4 000 m) would produce electrocardiographic signs of myocardial ischemia in young male subjects. It was realized that important differences probably exist between true myocardial ischemia during exercise in a patient with coronary disease and the condition likely to result from experimentally induced hypoxia. Myocardial ischemia implies a retention of carbon dioxide that probably does not occur during hypoxia in the absence of significant coronary artery disease. Hypoxia induces pulmonary hypertension and an increase in pulmonary vascular resistance (von Euler and Liljestrand 1946, Motley et al. 1947, Storstein 1958). Patients with coronary disease have a normal pulmonary vascular resistence during exercise (Malmborg 1964).

Material

Six male students in physical education volunteered to participate in the experiment. Anthropometric data are presented in Table I. The subjects were all well trained. All presented normal findings on physical examination. The medical history did not in any case suggest presence of cardiovascular, acute infectious, or other disease.

Pulmonary function tests including determination of forced vital capacity, forced expiratory volume and maximal ventilatory capacity gave normal results. The hemoglobin was normal (> 14 gm. per cent).

Methods

Exercise was performed on a mechanically braked *bicycle ergometer*. The *altitude chamber* has been described by Åstrand (1954). *Oxygen uptake* was determined by the Douglas bag method. Expired air was analyzed with a modified Haldane apparatus. *Intra-arterial blood pressures* were determined with an Elema-Schonander strain gauge and electromanometer and recorded on a Honeywell Visicorder. The system was calibrated against a mercury manometer at sea level and at the simulated altitude of

4,000 m. The third intercostal space at the sternum was used as a reference level. *Oxygen saturation* in arterial blood was determined according to Nahas spectrophotometric method as described by Holmgren and Pernow (1959) and *hemoglobin* on a Beckman B spectrophotometer. *Arterial* p_{O_2} was calculated from oxygen saturation and pH.

Arterial p_{CO_2}, *standard bicarbonate, base excess, and pH* were determined with an Astrup apparatus and calculated according to Siggaard Andersen et al. (1958). *Blood lactate* was analyzed according to Baker's and Summerson's method as modified by Ström (1948).

ECG were recorded and analyzed according to Section I. *Heart rates* were determined from the ECG.

Procedure

Preliminary studies were carried out at sea level and at simulated altitude of 4,000 m to define in each individual work loads which would produce exhaustion in about six minutes and cause an increase in arterial lactate levels to above 100 mg per cent.

Actual experiments were performed in the morning. The first study included exercise at simulated altitude of 4,000 m. and the second was control study at ground level. The interval varied between 4 and 15 days. The subjects were allowed to eat a light breakfast 2 hours before the experiment. Room temperature ranged between 18 and 22 centigrades.

A brachial artery catheter was inserted percutaneously. Frank lead ECG electrodes were applied. At the first experiment blood pressures and ECG were recorded twice during rest, the first time before the pressure in the altitude chamber was lowered to 462 mm Hg corresponding to an oxygen content of approximately 12 per cent. The rest of the procedure was identical at both experiments.

The subject rested for 20 minutes sitting comfortably in a chair and for another 10 minutes sitting on the bicycle ergometer. ECG and blood pressures were recorded, and a arterial blood sample was drawn.

The subject then performed leg exercise in sitting position for 6 minutes. The work load had been determined individually to correspond to 60 per cent of maximal aerobic capacity at either altitude. ECG was continuously monitored on an oscilloscope. Blood pressures were recorded after five minutes. Expired air was collected during the sixth minute and an ECG was recorded simultaneously. An arterial blood sample for blood gas and lactate analyses was drawn immediately before cessation of work. ECGs were recorded immediately and three minutes after exercise. A blood sample for lactate analysis was also drawn at three minutes after work. The exercise procedure was then repeated at a maximal work load after a rest period of 10 to 15 minutes.

Results

All subjects manifested marked cyanosis during hypoxic work. No untoward symptoms or signs were observed. There were no arrhythmias except moderate degrees of sinus bradycardia and sinus arrhythmia.

Circulatory and metabolic changes during hypoxic work will be discussed only in relation to electrocardiographic findings. Details from a series of experiments including this study will be reported by Stenberg (to be published). Table II presents a summary of the results in the present group.

Table I Anthropometric Data and Maximal V_{O_2}

No	Age	Height	Weight	B.S.A.	Maximal oxygen uptake L/min. Sea level	4,000 m
80	31	169	60	1.69	3 78	3 15
81	25	184	75	1 97	4 47	3 51
83	24	178	71	1.88	3 72	83
84	24	182	70	2.01	[illegible]	3.24
85	25	176	66	1.81	4.38	3.50
86	23	176	69	1.85	3 72	2.90

ECG Analysis

The interindividual variability was large with regard to all ECG variables There were no statistically significant group dif ferences bet ween work during hypoxia and the control study but certain trends were manifested.

ECG at rest A decrease of the oxygen tension from 123 4 to 45 6 mm Hg and of the arterial oxygen saturation from 97 9 to 81 2 with a small but significant increase in pH and decrease in carbon dioxide tension affected the ECG very little. There was a slight decrease of the mean spatial magnitude of the maximal T vector from 0 76 to 0 71 mV with no change in orientation. Correspondingly small changes were found throughout the ST T segment. There were no P or QRS changes and only minor differences in P R, ST and Q T durations The mean heart rate increased from 59.8 to 73 8

ECG during work at 60 per cent of maximal aerobic capacity As indicated by Fig 23 there was a larger interindividual variability in the ECG response during hypoxia than at sea level. There were no differences in the P and QRS segments. The magnitude of the frontal plane QRS-ST junction vector (QRS 8) was larger during hypoxia, but the most marked differences during the ST T segment were found at ST T 2 at the middle of the ST segment. This vector was at ground level directed to the left, downwards, and anteri orly During hypoxia there was a small rightward-upward displacement with no change in saggital direction, but ranges were overlapping The mean spatial magnitude of the maximal T vector remained essentially unchanged, 0 39 mV during hypoxia compared to 0 41 mV at sea level. The P R interval was shorter during hypoxia, 0 13 versus 0 15 seconds but the differences were not significant. ECGs recorded immediately after and 3 minutes after work showed smaller differences of the same type as those during exercise

No subject had any junctional depression exceeding 0 10 mV in lead X or 0 12 mV in lead Y immediately or 3 minutes after hypoxic work. The spatial magnitude of the maximal T vector increased to 0 61 mV immediately after hypoxic work, and to 0 59 mV at sea level Corresponding values 3 minutes after work were 0 53 and 0 59 mV Table II indicates that there was a marked reduction in oxygen tension and saturation during hypoxic exercise at the 60 per cent

Table II Physiological Data

	Rest		60 per cent		Maximal	
	Control	4,000 M.	Control	4,000 M.	Control	4,000 M.
Oxygen uptake, l/min STPD	—	—	2.38±0.06 2.17—2.49	1.82±0.11 1.54—2.17	4.05±0.14 3.72—4.38	5.19±0.22 2.83—5.51
Per cent of VO_2-max	—	—	58.9±0.7 58—62	51.2±2.5 47—62	100 %	—
Ventilation, l/min BTPS	—	—	50.4±3.2 43.1—75.8	51.1±5.7 41.8—74.4	124.6±5.6 110.4—143.6	148.6±10.2 102.0—165.2
Heart rate	52.9±2.3 45—59	71.5±5.7 51—95	134.3±3.1 124—148	138.5±7.8 110—157	187.0±2.3 183—196	180.2±3.1 175—191
Blood pressure, systolic	130.6 116—155	118.0 107—131	183.3 165—207	130.2 120—159	200.0 198—209	153.5 138—185
Blood pressure, diastolic	78.6 73—87	70.1 64—81	85.0 73—92	75.0 69—81	93.7 91—96	89.0 80—98
Blood pressure, mean	97.0 90—103	89.7 78—9	120.0 111—132	97.5 96—106	133.7 131—135	116.7 111—127
Oxygen saturation, per cent	97.9±0.4 98.8—97.1	81.2±1.4 77.2—86.5	96.9±0.6 91.1—98.6	70.8±1.5 66.5—76.1	97.1±1.7 96.3—98.8	69.9±1.0 66.4—71.8
P_{O_2} mm Hg	123.4±4.3 150—96	45.6±1.7 40—51	109.8±9.5 80—149	57.3±0.7 55—41	126.7±11.6 102—181	42.0±0.5 40—44
P_{CO_2} mm Hg	40.7±0.9 38—45	34.0±1.9 28—38	42.0±1.1 34—44	30.7±1.5 28—36	27.5±0.5 22—36	21.7±1.1 18—23
pH	7.40±0.003 7.39—7.42	7.44±0.01 7.42—7.48	7.36±0.01 7.33—7.43	7.42±0.01 7.39—7.45	7.26±0.01 7.25—7.33	7.27±0.02 7.19—7.24
Base excess	0.8±0.4 −1 — +2	0.3±0.7 −3 — +2	−2.7±0.5 −5 — 0	−3.0±0.5 −5 — −1	−13.8±1.5 −20 — −10	−15.3±1.1 −21 — −11
Standard bicarbonate	24.3±0.2 24—25	23.7±0.5 22—25	22.3±1.0 21—24	21.5±0.4 21—23	14.7±0.9 11—17	13.3±0.9 11—16
Lactic acid, mg per cent	10.7±1.1 5—14	—	30.6±7.1 7—42	56.5±4.4 23—52	118.7±11.2 83—161	158.7±10.2 130—196

The first row gives the group mean and standard error, the second the range. N = 6.

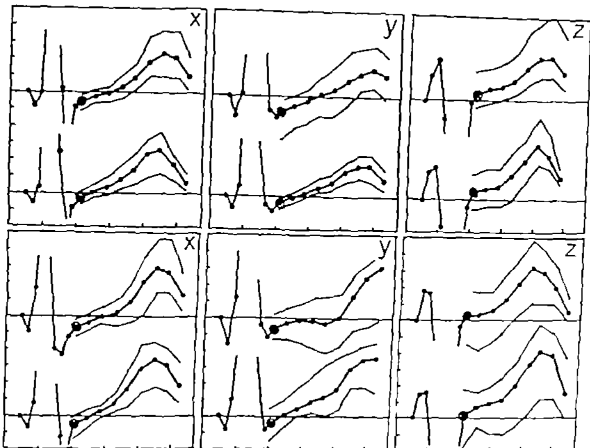


Fig 23 Mean 8/8 ST T amplitudes x y z among normal subjects during moderate and heavy work during acute hypoxia

Vertical scale divisions 0 1 mV horizontal 50 msec.
Thin lines indicate the range N = 6.
Top row ECG during exercise at simulated altitude of 4,000 m at a load corresponding to 60 per cent of individual aerobic capacity
Second row control study at sea level, 60 per cent relative load
Third row ECG during maximal exercise at 4,000 m.
Bottom row control study at sea level, maximal load.

level pH was 7 42 during hypoxia and 7 36 during the control study p_{CO_2} was somewhat lower during hypoxia (in both cases 0 05 > p > 0 02) Heart rates and arterial lactate concentrations did not differ significantly Ventilation (BTPS) was higher during hypoxia.

ECG during maximal work The ECG changes during maximal work were similar to those at the 60 per cent level but were more marked during the midd le part of the ST T segment. Intragroup ECG variability was higher during maximal work. Group differences between hypoxic and control maximal work in individual orthogonal leads were not statistically signi ficant, and generally smaller than at the 60 per cent level. However the lower range indicated in Fig 23 shows that rightward displacement of the entire ST segment did



Fig. 4 X, Y and Z lead (a) during and (b) three minutes after maximal hypoxic work
Subject No. 81 Vertical scale divisions correspond to 0.2 mV horizontal divisions to 100 msec.

occur within the group during hypoxia. The mean ST T vector 4 was directed upwards, corresponding to a frank inversion of the early part of the T wave in the vertical lead. Mean maximal T vector X and Z components were smaller during hypoxia, 0.31 and 0.30 mV versus 0.36 and 0.40 mV. Y lead amplitudes were largely influenced by atrial activity. There were no differences with regard to P-R, QRS, ST and Q-T durations.

Analysis of the ECGs immediately and 3 minutes after work showed decreasing differences between hypoxia and normal conditions. Only in one subject (No. 81 Fig. 24) were ST T vectors 2, 3, and 4 still directed to the right 3 minutes after exercise. A junctional depression exceeding 0.10 mV in the X and the Z lead occured in one subject (81) during maximal work, and in 3 subjects in the Y lead.

There was a small further decrease in mean oxygen saturation during maximal work compared to the 60 per cent level, but an increase in oxygen tension. There was also a significant decrease in pH but mean pH was essentially the same at bot maximal experiments, 7.27 and 7.26.

Correlation between individual metabolic and electrocardiographic findings. No wholly consistent pattern could be identified in an attempt to correlate metabolic and electrocardiographic changes. The same subject (No. 81) defined the lower extreme of the ST segment at both work load levels during hypoxia (Fig. 23 and 24). At the 60 per cent level he probably was

working at a higher relative load than any other member of the group as indicated by measurements of oxygen uptake. With regard to all variables studied except oxygen saturation at 60 per cent he demonstrated a more marked response to hypoxic work than the average member of the group but defined the lower range of the group only with regard to oxygen tension during hypoxic work. The subject (No 86) with the smallest ST deviation during maximal hypoxic word had the highest P_{O_2} of the group 44 mm Hg, and also an oxygen saturation above group average.

The subject (No 80) who had the highest lactate concentration, 179 mg per cent, and the most marked metabolic acidosis, pH 7 19 had a ST T segment closely corresponding to group average.

Comparison with ECG changes in patients with angina pectoris Sets consisting of QRS vectors at 6/8, 7/8 8/8 and ST T vectors at 1/8 2/8 6/8 at rest, during, and immediately and 3 minutes after work were compared with ECGs from patients with angina pectoris and from the older control groups in the linear pattern classification system described in Chapters V and VII.

ECGs recorded during hypoxia were all classified as belonging to the normal group except in subject 81 whose tracings were borderline at woth work load levels. All ECGs from the control study were classified as normal. The quantitative results of the analysis, expressed as individual distance to the hyperplane separating the patients with angina pectoris and the older control group suggest that hypoxic work induced ECG changes which moved the group average score during hypoxia closer to that of angina patients, but the response was within the normal range. The mean distance was —98.2 units at the 60 per cent load under normal conditions and —55 4 units during hypoxia. Corresponding figures for maximal work were —128 0 and —80 6 Individual variations conformed to the group pattern except in two cases in which the distance to the separating hyperplane did not change with hypoxia.

Discussion

Findings at rest

The abscence of ST depression in the resting ECG in young normal subjects during moderate hypoxia is in agreement with earlier reports e.g Larsen (1938) Levy et al. (1941) Biörck (1946) Malmström (1947) Penneys (1958) and Simonson (1961) The literature has been reviewed by Malmström and Simonson.

Penneys found a direct relationship between the ECG changes and arterial oxygen saturation at levels of 80 75 and 70 per cent. Simonson found no correlation between drop in 0_2- saturation and decrease in T wave amplitude in a sample of 58 normal young men studied at 0_2- saturations between 65 and 95 per cent, but a significant correlation in a group of 69 older men.

Data on pH, P_{O_2} and P_{CO_2} are lacking in many studies, and it is known that differences in degree of hyperventilation induced by the test procedure may cause differences in P_{O_2} not parallelled in oxygen saturation determinations (Malmström 1947) Most authors e.g Simonson (1961) state that ECG changes with hypoxia are related to P_{O_2} rather than to oxygen saturation. Christensen and Krogh (1936) first demonstrated that the appearance of clinical intolerance symptoms in normal subjects is determined by the alveolar oxygen tension level.

Findings during exercise

Little is known about the effect on the ECG in young normal subjects of the combined stresses of exercise and hypoxia. Akesson and Malmström (1945) studied 25 normal subjects, 24 to 44 years old, and 13 patients with angina pectoris a) after moderately heavy exercise b) at rest during hypoxia, and c) after light exercise during hypoxia. The exercise consisted of a step test increasing the oxygen uptake to 2 to 3 times the resting value. The load during hypoxia was equal to half the load under normal conditions. ECGs were recorded 2 minutes after work. The following changes were found in the control group (a) produced a small decrease in T wave amplitude, and (b) gave a moderate reduction ranging from 0.15 mV in lead I to 0.30 mV in chest lead IV (c) caused a slight further decrease af approximately 0.05 mV Only one of the subjects in the normal group showed a ST depression exceeding 0.12 mV after (c) There were no ST changes after (a) or (b) and no T wave inversion in lead II among the controls. The authors concluded that the subject developing significant ST depression after (c) probably should not have been included in the control group and that an ST depression exceeding 0.12 mV after work during hypoxia should be regarded as pathological The shape of the ST segment was not considered.

It is difficult to compare our results with Akesson and Malmström findings not only because of differences in lead systems. Their external work load was probably lower than the 60 per cent load in this series. The arterial oxygen content was not determined. Excluding their single questionable control subject they apparently did not in normal subjects find any ST changes suggesting ischemia. The degree of junctional depression corresponded approximately to our findings after exercise at the 60 per cent level.

The results of this study are compatible with an effect on the repolarization process of a reduced oxygen tension of the arterial blood, but far from conclusive ST T changes during exercise as compared to the ECG at rest were more marked during hypoxic work at the maximal level than at 60 per cent, but differences between hypoxia and the control study were smaller during maximal work (Fig. 23) This could be interpreted as being due to a lower arterial oxygen tension at the 60 per cent level than at the maximal level during hypoxia. Available hemodynamic data do not allow any analysis of myocardial work and oxygen consumption. However it should be noted that the mean arterial pressure was lower during hypoxia, 81 per cent of the control value at the 60 per cent level and 87 per cent at maximum. Stenberg (to be published) has demonstrated that the maximal cardiac output does not change during acute hypoxia. His data also suggest that there were no major differences in cardiac output at the 60 per cent level.

Significant pulmonary hypertension may have been present during hypoxic work and caused repolarization changes, but no data are available on pulmonary artery pressures and flow during strenuous hypoxic exercise in normal subjects.

There was no apparent relationship between pH and ST T changes.

SUMMARY

Magnetic tape recording, averaging technic, and digital computer analysis have been employed in a quantitative study of the Frank lead exercise electrocardiogram.

The report has been divided into two sections. Section I deals with technical aspects of data acquisition and processing and Section II with the results of application of a recording and analysis system to the study of six small experimental groups of patients with angina pectoris and controls. A total of 980 ECGs were recorded and analyzed.

Section I

Orthogonal lead systems seemed to offer several advantages and few disadvantages compared to conventional leads as a basis for quantitative ECG studies. *Frank's lead system* was selected. The vertical lead was modified to facilitate recording during exercise. ECGs were recorded on magnetic tape.

A computer of average transients (CAT 400B) completed in one operation the first two steps of the analysis procedure, *analog to-digital conversion* and *averaging*. Recordings were digitized with a sampling rate of 200 per second and lead. Averaging reduces random noise, e.g. muscle potentials and base line variation during exercise, by a factor equal to the square root of the number of ECG cycles averaged. The amplitude of the residual noise after averaging was sufficiently low to permit computer analysis of the entire material, including 160 exercise electrocardiograms recorded at heart rate of 150 or higher. A special procedure was developed to provide the computer of average transients with the anticipating trigger pulse required to include also the P wave in the analysis. The records were stored on paper punch tape.

A medium-sized *digital computer* (SAAB D21) was used in all subsequent data processing and analysis procedures. The paper punch tape data from the computer of average transients were transferred to magnetic tape. The records were normalized with regard to amplitude and time.

A *wave recognition program* based on spatial velocity was designed to identify the beginning and end of the P, QRS, ST and T segment at heart rates varying between 45 and 200. The program proved satisfactory with regard to definition of QRS, ST and T but gross errors (exceeding 30 msec.) were present in 6 per cent of the ECGs with regard to the beginning of the P wave.

A *data reduction procedure* condensing each ECG into a series of 75 amplitude measurements preceded the analysis stage. Each PR, QRS, and ST-T segment was divided into 8 subsegments of equal duration. X, Y and Z lead amplitudes at the beginning of the P wave and at the end of each subsegment were used to characterize each ECG.

The subgroups of the material were too small to permit statistical analysis on a vector basis, i.e. three-dimensional analysis. *Conventional statistical methods* were applied to data from separate orthogonal leads.

A *linear pattern classification method*, Rosenblatt's error-correcting training procedure (ECTP), was used in intra- and inter-group comparisons. Theoretically the method will provide (a) information on the diag

nostic contribution of each ECG amplitude measurement, (b) a means of quantitative diagnosis by reducing a large number (series of 75 and 108 amplitudes from each individual were used) of amplitude measurements into a single numerical diagnostic index. No criteria have to be specified in advance. The procedure developes criteria during training period. The performance of the method was evaluated in Section II.

Section II

ECG changes with increasing work loads in young and middle-aged male controls. Sixteen middle-aged men (range 45 to 56 years) and 11 young men (range 21 to 27 years) were studied at multiple work load levels including loads corresponding to 30, 60 and 100 per cent of individual aerobic capacity. QRS amplitude changes during work were similar in both groups with a right axis deviation and a change toward a more strictly posterior orientation of the mean QRS vector. ST-T changes were also similar at the 30 and 60 per cent level, but gross intergroup differences were present during maximal work with marked ST-T changes in the middle-aged men.

Both groups showed slight prolongation of the QRS duration with increasing work loads. A linear relationship between R-R interval and Q-T duration during exercise was demonstrated in both groups. There was strong relationship between heart rate and the magnitude of ST junction depression. The magnitude of the J depression appeared to be more closely related to the level of the T-P segment than to P wave amplitude.

ECG changes in patients with angina pectoris evaluated by means of the error-correcting training procedure. A selected group of 10 patients with angina pectoris was compared to the group of 16 middle-aged male controls. Series of ECGs at rest, during exercise, and immediately after and 3 minutes after exercise represented by 108 amplitude measurements in each individual series were analyzed by the error-correcting training procedure. Linear discriminant functions generated by the ECTP achieved complete separation of patients and controls at work load levels below and at the threshold of anginal pain. The discriminant functions were validated by observing the results of the classification procedure in similar ECG series and by comparisons with the results of numerical analyses. The repeat variability of the quantitative diagnostic index or score representing the result of the classification procedure was relatively low among the control, ± 7 per cent. A small subgroup of angina patients who were studied on three occasions showed a larger variability but score variations corresponded to differences in conventional ECG recordings.

The discriminant functions included series of weight factors, *one for each variable* considered in the analysis. The weight factors measure the relative significance of each variable. Analysis of the weight factors demonstrated that the ECG *during* exercise defined more than 40 per cent of the total difference between patients and controls at both work load levels versus 10—30 per cent immediately after and 3 *minutes after* exercise. The most important single measurement was the amplitude at 1/2 the distance end of QRS — end of T.

Patients with angina pectoris studied during work in cold environment and during arm work. Clinical and physiological data suggested that work in cold environment and arm work could represent experimental procedures to change the relationship between

myocardial oxygen demand and supply while keeping the work load constant in terms of total body oxygen uptake. Two small groups of 5 and 6 angina patients were studied. The cold experiment produced a somewhat higher heart rate and diastolic blood pressure than a control study. Three men also had more angina during the cold study and the group as a whole had also more marked ST T changes. Arm work produced a somewhat higher heart rate, mean and diastolic blood pressure than leg work, but symptoms and ECG changes were more marked during leg work.

ECG changes in normal subjects during strenuous hypoxic work. A group of six young normal subjects were studied during exercise at relative work load levels of 60 and 100 per cent of aerobic capacity at a simulated altitude of 4 000 m and at sea level. The ECG during hypoxic work at the 60 per cent level showed more ST T changes in comparison with the control study than did the ECG at the maximal level. The arterial oxygen tension was lower at the 60 per cent level. Only one subject showed some degree of horizontal ST depression.

A major part of the results of this study requires further support before definite conclusions can de drawn. The main findings can be summarized as follows

1 The experimental results demonstrate that electronic methods of data acquisition and processing greatly facilitate quantitative electrocardiographic studies.
2. Findings in patients with angina pectoris and in controls suggest that the ECG during exercise contains more information than ECGs recorded after exercise. Averaging technic is a definite asset in the analysis
3 The response to exercise was similar among young and middle-aged controls at light and moderately heavy work loads, but the middle-aged men showed definitely larger ST T changes at maximal loads. This finding suggests that ST T changes provoked by maximal o near maximal loads may not have the same prognostic significance as corresponding changes at lower work loads.
4. Linear pattern classification methods may provide means of converting large numbers of numerical measurements into concise, clinically useful information.

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SUPPLEMENTUM 439

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Studies with Neutron Activation Analysis

By

P O Wester

ACCOMPANIES VOL. 178

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P. O. WESTER

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STOCKHOLM 1965

From the Department of Medicine (Head G. Biörck) Karolinska Institutet at Serafimerlasarettet
and the Department of Chemical Research (Head E. Haeffner) AB Atomenergi, Stockholm, Sweden

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The present dissertation is based on the following five papers

I Wester P O Brune, D and Samsahl, K. Radiochemical recovery studies of separation scheme for 23 elements in biological material. Int. J Appl. Rad. Isot. 15.59 1964

II Wester P O Concentration of 24 trace elements in human heart tissue determined by neutron activation analysis. Scand. J Lab. Clin. Invest 357 17 1965

III Wester P O Concentration of 17 elements in subcellular fractions of beef heart tissue determined by neutron activation analysis. Biochim. Biophys. Acta. In print.

IV Wester P O Trace elements in the conductive tissue of beef heart determined by neutron activation analysis. Acta Med Scand In print.

V Wester P O Trace elements in human myocardial infarction determined by neutron activation analysis. Acta Med. Scand. In print.

Reference will be made to these papers using the Roman figures as listed above.

To my wife and children

STOCKHOLM 1965
KUNGL. BOKTRYCKERIET P. A. NORSTEDT & SÖNER

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Introduction

The biological significance of trace elements

The occurrence of certain trace element in biological material has been known for a long time. Cu and Zn were detected in human tissues about 100 years ago (64). At that time, elements present in such small amounts were regarded merely as scientific curiosities, and believed to be contaminants. Since then, opinions have been revised, and many trace elements have been shown to be indispensable to various species of animals (6, 19, 59, 80, 91, 92). In man, some trace elements are known to be essential, e.g. Co and Fe, and others are considered to be essential, e.g. Cu and Zn. Several trace elements have suspected biological function, e.g. Ba, Br, Cd, Cr, Mo, Rb and Se, whereas most of those normally present in human tissues are still without known biological function. The trace elements of biological significance function mainly as catalysts or activators in enzyme systems or other organic complexes. Hundreds of enzymes or enzyme systems are known to be influenced by trace elements (80). Certain enzymes may be activated by a number of different elements, whereas others — the so-called metalloenzymes — contain particular trace elements as an indispensable structural part. Examples of such specific metalloenzymes are ascorbic acid oxidase, butyryl CoA dehydrogenase, ceruloplasmin, cytochrome oxidase and tyrosinase containing Cu, as well as catalase, various cytochromes and DNPH cytochrome c reductase containing Fe, and xanthine oxidase containing both Fe and Mo. Alcohol dehydrogenase, carbonic anhydrase, carboxy peptidase, glutamic acid dehydrogenase and lactic dehydrogenase are examples of metalloenzymes containing Zn.

Besides the trace elements of biological significance, metabolic or anti-metabolic activities can be ascribed to a large number of trace elements, but their role, if any, in the normal or diseased human organism is poorly understood. However according to Butt (15) "the discovery of specific metalloenzymes, metal enzyme complexes, the localization of metals in cells and cellular constituents, and the acquisition of new and perfected tools for measuring metals in trace amounts, have added materially to the understanding of metal functioning in the normal and abnormal man".

Although most trace elements normally occurring in mammalian tissues have no known biological function, several authors consider that many of those now regarded as physiologically inactive will, with great probability, be found to participate in vital biological processes (23, 80, 92).

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Classification of trace elements

The only common property of trace elements is their presence in minute quantities in biological material. The demarcation from the elements occurring in large amounts, the so-called bulk elements, is not definite. Ca in bone and Fe in blood, for example, occur in amounts comparable with the bulk elements. The amount of Ca and Fe in other tissues, however, is within the range of the trace elements. In the present studies (II—V) a concentration of 100 parts per million has been used as a borderline between bulk and trace elements.

The various groupings of trace elements in different categories made by several authors have mainly a functional basis (62, 73, 74, 88, 89, 91). The grouping used in the present studies (II—V), i.e. into the trace elements with, with suspected and without known biological function, has no definite borderlines. With increasing knowledge of trace elements in biological material, the group with known biological function will probably increase to the detriment of the other two groups.

Although no exact dividing lines can be drawn between these groups, some kind of grouping from a functional point of view is, however, useful in discussion.

Trace elements in heart tissue

The available data on the occurrence of trace elements in heart tissue are chiefly limited to those obtained with spectrographic methods. Quantitative determinations of certain trace elements in normal human heart tissue have been performed by several investigators (e.g. 31, 40, 48, 50, 83, 84, 88, 89). Range and central values have been given for the trace elements with known biological function Ca, Co, Cu, Fe, Mn and Zn. Among the elements with suspected biological function, range and central values have been presented for Ba, Br, Cr, Mo and Rb. Cd has, however, been detected only in some of the samples investigated, and Se does not seem to have been determined. Among the elements without known biological function, data seem to exist only for Ag, Au, Cs and Hg, but not for As, Ce, La, Pt, Sb, Sc, Sm and W.

Quantitative determinations of trace elements in normal whole heart tissue of beef or other animals seem to be limited (49, 82). Investigations of trace elements on a subcellular level have been performed in liver tissue (44, 87), but appear to be lacking in heart tissue. The specialized heart tissue in the conducting system of beef heart has been investigated with respect to certain trace elements, and semiquantitative values of some elements have been reported (41).

Quantitative determinations of some trace elements in human heart tissue in certain diseased states, e.g. diabetes mellitus, haemochromatosis and liver cirrhosis (17, 36), have been presented. Data on the concentration of trace elements in diseased human heart tissue seem, however, to be scanty (34, 36, 57).

Methods of trace element analysis

Various methods have been used for determination of trace elements in biological material, e.g. different spectrographic methods, colorimetry, amperometric titration and X-ray fluorescence

Emission spectrography seems mostly to have been used in previous studies. In recent years, neutron activation analysis has attracted increasing interest, because of its extremely high sensitivity for all but a few elements (53) The high sensitivity of activation analysis compared to other methods is apparent from a compilation made by Meinke (56) In the present studies, neutron activation analysis has been used mainly on the grounds of its high sensitivity The almost complete absence of risks of reagent contamination after irradiation also makes this method advantageous for the determination of elements in trace quantities. In the following, a short account is given of neutron activation analysis, with special reference to the analysis of trace elements in mammalian tissues.

Neutron activation analysis

Neutron activation analysis, introduced in 1936 by de Hevesy and Levi (26) has been extensively utilized in recent years, mostly in determination of trace elements. Several books on the subject have been published (e.g. 14 37 38) as well as numerous articles (12, 52)

The material to be investigated is irradiated with elementary particles, chiefly neutrons, or γ-rays of high energy The nuclides in the material then become radioactive, and from the activity induced it is possible to determine several of the elements present in the material.

In stable nuclides, the protons and neutrons have a certain mutual relation. Irradiation, e.g. with neutrons, may alter this relation, generally giving rise to unstable (radioactive) nuclides. Every radioactive nuclide emits a specific radiation, e.g. α- β- and γ-radiation, which is characterized by certain energies (monoenergetic for α and γ) and a certain half life. Certain radioactive isotopes are naturally occurring, e.g. C[14] K and Ca[14] but most are induced by irradiation.

The following description is confined to the irradiation in an atomic reactor which is the kind of activation mainly used in this connexion. The neutron flux produced in a reactor contains neutrons of different energies, which may cause various neutron reactions. The (n, γ) reaction is the most prominent one at low energy of the neutrons (thermal neutrons ~ 0.03 eV) With increasing energy of the neutrons, the cross-section (the probability that a reaction will occur expressed in barns) decreases for the (n, γ) reaction. Some exceptions are present in the epithermal region (~ 0.2 eV — ~ 0.05 MeV) Fast neutrons (> 0.5 MeV) may cause other reactions, e.g (n, p) (n, α) and (n, 2n) The cross-sections of these reactions are generally exceedingly small, at least 10-fold smaller than those of the (n, γ) reactions. In some cases, these reactions may advantageously be used in determination of certain elements (12) However they often only constitute a source of error by interfering with the main (n, γ) reaction.

The quantity of radioactivity induced in material during irradiation is given by the following equation

$$I = \frac{W \cdot \theta \cdot N \cdot \sigma \cdot \Phi}{A} (1 - e^{-\lambda t}) e^{-\lambda t}$$

where I = the activity in disintegrations/second

—67396 Wester

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where I = the activity in disintegrations/second

1—671246 Weber

θ = isotopic abundance of the reacting nuclide

W = mass of the element in grammes

Φ = thermal neutron flux in neutrons/cm²/second

σ = activation cross-section of the reacting nuclide of the element

N = Avogadro's number $6.02 \cdot 10^{23}$

A = atomic weight of the element

$(1-e^{-\lambda t})$ = the saturation factor where λ is the decay constant (equal to ln 2 divided by the half life of the induced radionuclide) and t is the irradiation time

$e^{-\lambda T}$ = a factor which represents the decay of the activity after irradiation where λ is the decay constant and T is the time that has elapsed after the end of irradiation

If the activity is known, the concentration of an element present in the sample can be obtained by solving W in the equation. In practical work it is, however, more convenient and even more accurate to use a comparative method, since e.g. the neutron flux seldom is exactly known and furthermore may vary during irradiation.

Known amounts of the elements to be determined (standards) are irradiated together with the sample. The activities induced in the sample are compared to the activities of the corresponding standards, and the amounts sought are calculated by the rule of three.

In view of the sensitivity of activation analysis, this method is very advantageous for the determination of elements in trace quantities, especially if the amount of sample material is limited, as is often the case in biological samples. Activation analysis may also be favourable for the determination of elements occurring in large amounts in biological material, e.g. the electrolytes, if the samples available are small, e.g. needle biopsy samples (5). Using activation analysis, the main components of biological material — C, H, O and N — do not interfere with the determination of the other elements, since the nuclides formed from them either have very faint activities or short half lives.

The main activities formed by neutron irradiation of mammalian tissues emanate from Cl^{38}, Na^{24} and P^{32}. They almost completely cover all other activities formed, and some kind of elimination procedure is necessary to allow measurement of the activities behind. Cl^{38} with the half-life of 37 minutes, is a problem if the elements to be determined have only short lived nuclides. Otherwise, the irradiated sample may cool to allow the activity of Cl^{38} to decay. Na^{24} with the half life of 15 hours and the high γ-energies of 1.37 and 2.75 MeV is a major problem, and must be completely eliminated to permit measuring several of the nuclides formed from the trace elements occurring in tissues. P^{32} with the half life of 14.2 days, is a pure β-emitter. Its bremsstrahlung disturbs the measurements of nuclides of low γ-energy and must mostly, if the elements to be determined have only nuclides of low energies, also be eliminated.

There are two essentially different ways of performing neutron activation analysis, one non-destructive and one involving chemical separations. Using the non-destructive method, some means of discrimination between the different activities usually present in the irradiated

sample must be available. The rapidity of this method is advantageous, but the sensitivity is often far lower than that involving chemical separation. Chemical separation can also be performed in two essentially different ways. One is to determine one or a few elements at a time by precipitation or some other rapid analytical procedure. Several methods in which such a technique is used have been devised (e.g. 13 31). Another way is to determine a great number of trace elements simultaneously in one sample by some group separation system. Some such systems have recently been developed (2—4 32, 33 67—70).

The elements are identified with the aid of the energies and half-lives of the radionuclides formed. The γ-quanta emitted are usually detected by a suitable scintillator generally a NaI (Tl) crystal, attached to a multichannel pulse-height analyzer and the γ-spectrograms obtained can be studied e.g. in an oscilloscope. Typical γ-spectrograms obtained from irradiated and separated human heart tissue are seen in the Appendix (diagrams 1—14).

When using neutron activation analysis, several sources of error must be considered. Great care must be taken in preparing the samples before irradiation, to avoid contamination or losses of volatile elements. During irradiation flux gradients, flux depression and self-shielding may cause inhomogeneity of the neutron flux. A prerequisite is that the sample and standard are subjected to the same neutron flux.

In an atomic reactor however the thermal neutron flux is usually greatest in the centre and decreases towards the periphery. The degree of the flux variation per unit of length — the flux gradient — varies in different positions in the reactor. The error due to flux gradients can be reduced by choosing a position with a low gradient, and by placing the sample and the standard as close as possible to each other. Elements of high cross-section near to or in the sample or standard may attenuate the flux, and thus prevent the sample and the standard from receiving the same flux — so-called flux depression. Consequently a check should always be made of irradiation positions close to the positions used, to ensure that they do not contain any strong neutron-absorbing material. If the sample contains components of high neutron cross-section, the neutron flux at the centre of the sample will be less than at the surface so-called self-shielding, and the average flux to which the sample has been subjected therefore differs from that of the standard. In tissue samples, however elements of high cross-section occur in such low quantities that this effect may be neglected, particularly if the samples are small. The self-shielding effect in the standards can be reduced by using minute quantities.

The possibility of errors due to interfering neutron reactions caused by fast neutrons has been pointed out in the aforegoing. The cross-sections of these reactions are in general extremely low and the parent nuclides in question occur in most tissue samples in such small quantities that interference with the main (n, γ) reaction may be neglected. A correction must, however be made for the contribution of some interfering reactions. For example, in determining

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There are two essentially different ways of performing neutron activation analysis, one non-destructive and one involving chemical separations. Using the non-destructive method some means of discrimination between the different activities usually present in the irradiated

sample must be available. The rapidity of this method is advantageous, but the sensitivity is often far lower than that involving chemical separation. Chemical separation can also be performed in two essentially different ways. One is to determine one or a few elements at a time by precipitation or some other rapid analytical procedure. Several methods in which such a technique is used have been devised (e.g. 13, 51). Another way is to determine a great number of trace elements simultaneously in one sample by some group separation system. Some such systems have recently been developed (2—4, 32, 33, 67—70).

The elements are identified with the aid of the energies and half lives of the radionuclides formed. The γ-quanta emitted are usually detected by a suitable scintillator, generally a NaI (Tl) crystal, attached to a multichannel pulse height analyzer and the γ-spectrograms obtained can be studied e.g. in an oscilloscope. Typical γ-spectrograms obtained from irradiated and separated human heart tissue are seen in the Appendix (diagrams 1—14).

When using neutron activation analysis, several sources of error must be considered. Great care must be taken in preparing the samples before irradiation to avoid contamination or losses of volatile elements. During irradiation, flux gradients, flux depression and self-shielding may cause inhomogeneity of the neutron flux. A prerequisite is that the sample and standard are subjected to the same neutron flux.

In an atomic reactor, however, the thermal neutron flux is usually greatest in the centre and decreases towards the periphery. The degree of the flux variation per unit of length — the flux gradient — varies in different positions in the reactor. The error due to flux gradients can be reduced by choosing a position with a low gradient, and by placing the sample and the standard as close as possible to each other. Elements of high cross-section near to or in the sample or standard may attenuate the flux, and thus prevent the sample and the standard from receiving the same flux — so-called flux depression. Consequently, a check should always be made of irradiation positions close to the positions used, to ensure that they do not contain any strong neutron-absorbing material. If the sample contains components of high neutron cross-section, the neutron flux at the centre of the sample will be less than at the surface, so-called self-shielding, and the average flux to which the sample has been subjected therefore differs from that of the standard. In tissue samples, however, elements of high cross-section occur in such low quantities that this effect may be neglected, particularly if the samples are small. The self-shielding effect in the standards can be reduced by using minute quantities.

The possibility of errors due to interfering neutron reactions caused by fast neutrons has been pointed out in the aforegoing. The cross-sections of these reactions are in general extremely low and the parent nuclides in question occur in most tissue samples in such small quantities that interference with the main (n, γ) reaction may be neglected. A correction must, however, be made for the contribution of some interfering reactions. For example, in determining

the P content of tissues by P^{31} (n, γ) P^{32} the reaction S^{32} (n, p) P^{32} must be considered, and the reaction Fe^{56} (n, p) Mn^{56} must not be overlooked in the determination of Mn in blood by Mn^{55} (n, γ) Mn^{56}

Interference by activation of the radionuclide formed may sometimes occur e g in the determination of Pt in tissues by Pt^{198} (n, γ) Pt^{199} Pt^{199} $\xrightarrow{\beta^-}$ Au^{199} the formed Au^{199} also derives from Au^{197} (n γ) Au^{198} Au^{198} (n γ) Au^{199}

In the determination of some nuclides in tissues, e g Cd^{115} Ce^{141} La^{140} Mo^{99} and Sm^{153} interference by neutron induced fission reactions (n, f) must also be taken into account if the samples contain uranium.

Losses of volatile elements may be prevented by irradiating the samples in sealed ampoules (99) Errors introduced in γ-spectrometric measurements can be reduced if complex γ-spectra are avoided e g by chemical separation, and *if the* counting rate is high enough to make the statistical uncertainty small.

Thus, most of the possible errors can be avoided or reduced, and the precision in neutron activation analysis is considered to be $\pm$ 10 % (55) or better (20, 37)

Present investigation

The known biological importance of some trace elements and the suspected significance of others, as well as the lack of available data on the concentration of several trace elements in both normal and diseased heart tissue, have been pointed out in the aforegoing

The intimate association between trace elements and enzymes has also been related. In recent years, enzymic studies have been made of various tissues, particularly those of the heart and liver

The scanty information about the *concentration of several elements in whole heart tissue and the almost complete lack* of trace element data on the subcellular level and in the conductive tissue where only small samples are available, are obviously due to the limited sensitivity of the methods of trace element analysis mainly used in earlier studies.

The present investigation was undertaken to fill some of the gaps existing in our knowledge of trace elements in heart tissue.

The aims of the investigation can be *specified as follows*

1 To apply a recently developed technique to the determination of trace elements in biological material.
2 To investigate the occurrence of trace *elements in normal human heart* tissue, and to define their range of concentration.
3 To investigate the occurrence of trace elements in beef heart tissue, and to compare the results *with those* obtained in human heart tissue.
4 To investigate the trace element distribution on a subcellular level in beef heart tissue.
5 To investigate *the occurrence of trace* elements in the specialized heart tissue in the conducting system *of the* beef heart.
6 To investigate the concentration of different trace elements in injured and uninjured heart tissue from patients who died of *myocardial infarction*.

PART I

Methods

Preparation of samples

Using neutron activation analysis, the risk of contamination of the samples after irradiation is practically non-existent. Before irradiation, however great care must be exercised to avoid contamination. In the dissection of tissue, only glass or plastic instruments were used, and no contact between the samples and metallic objects was allowed. All the glass and plastic instruments used were thoroughly cleaned before use with aqua regia or 6 N HCl and demineralized water. The samples were transferred to cleaned quartz ampoules (10 mm diameter 60 mm height) and dried. The possibility of losing volatile elements during the drying step was studied (II). Losses were detected only for Hg, which did not, however exceed 15 %. After sealing with a flame, the ampoules were ready for irradiation with thermal neutrons in an atomic reactor.

Neutron irradiation

Samples and standards were always irradiated simultaneously. Most of the irradiations were performed in the Reactor R2 at Studsvik, with a thermal neutron flux of 2 10^{13} n/cm sec for 24—75 hours. Some of the irradiations were performed in the Reactor R1 in Stockholm, with a thermal neutron flux of 2 10^{12} n/cm sec for 24—75 hours. Usually two samples and 9—10 standards were placed in one irradiating can, and the remaining standards in another irradiating can together with an additional Zn standard, to correct for neutron-flux variations. The positions close to the irradiation positions used were checked to ensure the absence of any strong neutron-absorbing material. The irradiated samples were allowed to cool for 1 to 3 days before chemical separation, in order to reduce the very high Na activity.

Preparation of standards

From carefully prepared weakly concentrated stock solutions of the elements to be determined, 50—100 µl were pipetted into small quartz ampoules (4 mm diameter 50 mm height). The ampoules were dried and sealed. Studies were made to ensure that no losses of volatile elements occurred during the drying step (71).

Chemical separation

Chemical separation was performed with recently developed ion-exchange technique combined with subsequent γ-spectrometry (68—70). The applicability of the technique to biological material was investigated (I) and in this connexion studies were made of the recovery and reproducibility

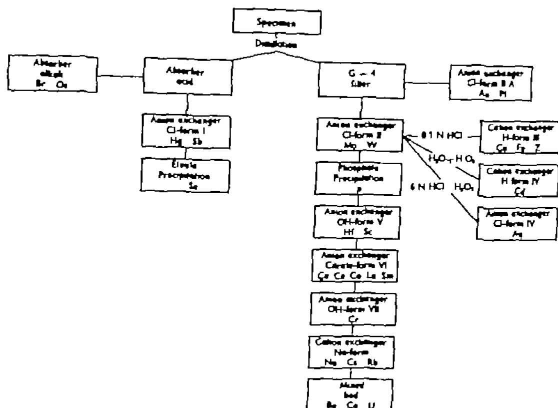


Fig 1 Distribution of the different elements among the different ion-exchange resins and precipitations.

The first step in the chemical separation method involves destruction of the irradiated organic material in a closed system by charring with H_2SO_4 and oxidizing the carbon with H_2O_2. Volatile elements are then distilled off by addition of HBr. Most of the further separations are performed by ion-exchange steps, of which an important part is based on adsorption and elution of chloride complexes on anion exchange resins (1). Carriers are added since a precipitation step (phosphorous as zirconium phosphate) is included in the separation system. Fourteen different groups well suited for γ-spectrometry are obtained each containing nuclides originating from 1 to 3 elements. One group contains nuclides from 5 elements these are, however easy to determine, owing to the various half lives of the nuclides considered for measurement. The distribution of the different elements among the different ion-exchange resins and precipitations is seen in fig 1.

In the recovery studies (1) known amounts of activities of the different elements to be investigated were mixed with pieces of heart or liver tissue. The same amounts of activities from the same standard solutions were used as reference activities. The activities which had passed the separation system were then compared to the corresponding reference activities. A recovery value of > 90 % was obtained for all elements studied except

Au and Se (84 and 80 respectively). The standard error of the mean was ≤ 3 % for all the elements studied.

Gamma ray spectrometry

The gamma-spectrometric measurements were carried out with a transistorized 512-channel pulse-height analyzer attached to a 3" × 3" NaI (Tl) well-type crystal. The samples were counted either close to or inside the well of the crystal, immediately before the corresponding standards. The standards were counted at suitable distances from the crystal. Correction factors were determined for the differences obtained by counting the samples and the standards under different geometrical relations (II).

The elements were identified both by aid of the energies and of the half-life of the formed nuclides (I, II). Moreover additional confidence in the identification was afforded by the fact that the different elements investigated are to be found on definite ion-exchange resins or precipitations in the chemical separation system used.

Precision and accuracy

Great care was taken to avoid contamination of the samples, especially before irradiation but after irradiation as well, precautions were taken to prevent cross-contamination between irradiated samples or standards.

The sources of error inherent in the activation process, such as self-shielding, flux depression, flux gradients and interfering reactions, were taken into account. Possible errors introduced by the chemical separation system were investigated, and corrected for (I). The possibility of losing volatile elements was avoided by irradiating the samples in sealed ampoules, and by using a closed chemical separation system. Losses of Hg did, however, occur during the drying step of the samples before irradiation. No appreciable error can be assumed in evaluation of the γ-spectrograms obtained. The statistical uncertainty in the counting rate cannot, on the other hand, be disregarded.

Although the uncertainty of the results is difficult to estimate correctly, it undoubtedly exceeds the uncertainty deriving from the counting rate. Nevertheless, the accuracy of the results is probably better than ± 10 %, which has been regarded as a usual figure for the precision of neutron activation analyses (55).

PART 2

Trace elements in normal human heart tissue

Some systematic investigations of certain trace elements in normal human tissues have been made earlier (e g 40 48 50 83 84 88 89) and the amount of some such elements in human heart tissue has been determined. The available data are mainly limited to those obtained by spectrographic methods The sensitivity of these methods has not however sufficed for quantitative determination of several trace elements normally occurring in such tissue nor even for their detection

In the present work (II) the aim was, firstly to determine which trace elements are present in normal human heart tissue and, secondly to determine their range of concentration. Heart tissue, with no signs of macroscopical or microscopical damage, from autopsy of victims of traumatic accidents was considered to represent the closest possible approach to normal heart tissue

Human heart tissue (the anterior wall of the left ventricle) from autopsy of 20 victims of accidents (15 males and 5 females) ranging in age from 4/12 to 65 years was therefore investigated The following 24 trace elements Ag As, Au, Ba, Br Ca, Cd Ce, Co Cr Cs Cu, Fe, Hg La Mo Pt Rb Sb Sc, Se, Sm W and Zn were detected with the exception of Hg in one sample, whereas Hf and Os could not be detected regularly All elements except Hf and Os were quantitatively determined In agreement with the results of other investigations (40 90) most of the elements were found have a skew distribution. The median was therefore used as the central value, and the mean and standard deviation were given only for the elements Cu Fe, Se and Zn which appeared to have a normal distribution

Good agreement was, in general, found when comparing the trace element concentrations with those reported in other investigations (e.g 15, 31 35 40 48, 82—84 88, 89) Information about the concentration of As, Ce, La, Pt, Sb, Sc, Sm and W in normal human heart tissue does not however seem to be available in the literature Ag Au and Cd have not been detected regularly in earlier investigations. Thus, central values and lower limits of these elements were lacking for comparison

The trace elements investigated were divided into three groups, i e. those with, with suspected and without known biological function (cf page 8) In agreement with the results of Tipton and Cook (89) the concentration of the elements with known biological function except Co was found to show the least degree of variation Most of the elements without known biological function showed great variations, some of them almost excessive e.g Au and Sm, whereas the variation of the elements

with suspected biological function was, in general, something intermediate. The elements with known biological function are stated to be more ubiquitous than those belonging to the other groups (89). The question of ubiquity is, however probably dependent on the sensitivity of the investigation method, since all elements sought in the present investigation were always detected, except for Hg in one sample.

Obvious differences in the concentration of trace elements with age have been reported in comparing newborns with adults (e.g. 17, 83). After 3 months of age, however, such differences have been reported only for certain elements in certain tissues (e.g. 62, 73, 79, 83). In the present investigation (II) no significant differences with age were obtained. The As values of the oldest cases were somewhat higher than those of the youngest ones, but no significant difference was present ($p = 0.2$). Nor were any significant sex differences detected. Variations in the trace element concentration due to environmental conditions could be estimated only to a very limited extent.

Since all the cases investigated came from Stockholm with surroundings, geographical variations were not investigated. Nor could the variations, if any, due to different nutritional conditions be evaluated. With regard to occupation, high amounts of As and lanthanides were obtained in the heart tissue of one industrial worker.

Trace elements in beef heart tissue

Trace elements in whole tissue of beef heart

Beef heart tissue was found to contain the same elements in similar concentrations as human heart tissue. The concentration of some elements, however differed significantly. Table I shows a compilation of the results obtained from human whole heart tissue (II) and from whole heart tissue of beef heart (III and IV). Less Cd, Co, Se and Zn was found in the beef hearts than in the human hearts (significance***). A lower concentration of Co and Zn in beef heart tissue than in human heart tissue has previously been reported (49). The amount of Ag was also somewhat lower in the beef hearts than in the human hearts (significance*), whereas the amount of Ce was somewhat higher (significance*). The other 17 trace elements investigated did not differ significantly. The beef heart material comprised samples from 2 calves and 7 adult animals. No obvious differences could be discerned between the trace element concentration in the samples from the calves and those from the adult animals. Possibly the Hg values were somewhat lower in the former than in the latter. The samples from the adult animals consisted partly of a mixture of muscle tissue from the anterior and posterior wall of the left ventricle and partly of muscle tissue from the ventricular septum. No apparent differences were noted between the trace element concentrations in different parts of the left ventricle. The lowest concentration of Au and Cu was, however observed in the ventricular septum.

Trace elements in subcellular fractions of beef heart tissue

Trace elements of known biological significance function mainly as catalysts or activators in enzymes or enzyme systems. The distribution or the localization of various enzymes or enzyme systems to different cytoplasmic organelles has been thoroughly investigated. In this connexion, two different tissues, i.e. rat liver and beef heart tissue, have been studied extensively. Investigations of the distribution or the localization of trace elements in subcellular fractions are, however limited and apply only to some elements in rat liver tissue (44, 87). The aim of the present work (III) was therefore to investigate the distribution of a large number of trace elements in subcellular fractions of beef heart tissue.

The different subcellular fractions investigated, i.e. nuclear and cell residue fraction, mitochondrial fraction, sarcotubular fraction, and supernatant, were obtained by differential centrifugation in sucrose solution. The homogeneity of

TABLE I. Trace element concentration in human and beef whole heart tissue. Amounts in µg/g wet tissue.

	Human		Beef		
	Range	Median	Range	Median	Significance[1]
Ag	0.0006—0.025	0.0025	0.0002—0.005	0.0007	
As	0.00097—0.012	0.00236	0.0017—0.0071	0.0023	
Au	0.0000008—0.00011	0.0000338	0.000003—0.000085	0.000022	
Ba	0.007—0.03	0.020	0.004—0.03	0.007	
Br	1.0—4.6	2.03	1.1—3.6	2.1	
Ca	24—96	45.6	16—61	35	
Cd	0.01—0.03	0.012	0.0003—0.004	0.0009	
Ce	0.001—0.008	0.0016	0.002—0.01	0.004	
Co	0.001—0.018	0.0121	0.003—0.009	0.006	
Cr	0.0017—0.020	0.0062	0.0010—0.015	0.0028	
Cs	0.0064—0.022	0.0114	0.0041—0.022	0.0093	
Cu	2.0—5.2	3.75	2.4—4.8	3.8	
P	21—53	35.2	31—49	39	
Hg	0.009—0.096	0.0431	0.0029—0.23	0.033	
La	0.0001—0.003	0.00029	0.0002—0.001	0.0004	
Mo	0.026—0.13	0.0314	0.040—0.12	0.056	
Rb	1.7—3.6	2.45	1.8—3.3	2.6	
Sb	0.001—0.004	0.0015	0.0007—0.003	0.0018	
Sc	0.000003—0.0001	0.000014	0.000009—0.0001	0.000013	
Se	0.097—0.25	0.177	0.047—0.14	0.061	
Sm	0.0002—0.002	0.0025	0.0003—0.004	0.0019	
W	0.0007—0.002	0.0012	0.001—0.002	0.0012	
Zn	18—33	25.0	12—23	16	

[1] The statistical calculations for Cu, P and Zn were made by Student's t test and for the other elements by Wilcoxon test.

the mitochondrial and sarcotubular fractions was tested electron-microscopically and was considered to be satisfactory (III figs 1 and 2). The preparation of samples before irradiation involves, as pointed out in the aforegoing, great risks of contamination. Contamination of some elements, i.e. Ag, A Cd Ce Cr Sb and Sc did, in fact, occur in the present work (III) and these elements were therefore omitted from the study.

The concentration of the following trace elements in the different subcellular fractions from three cell fractionation experiments was investigated As, Ba, Br Ca, Co, Cs, Cu, Fe, Hg La, Mo Rb, Se, Sm, W and Zn. The bulk element P was also determined. The amounts were expressed in µg/g wet tissue as well as µg/g protein.

The concentration of each element in the same fraction from different hearts was much the same whereas the concentration in different fractions from the same heart varied.

The trace elements with known biolo-

PART 3

Trace elements in beef heart tissue

Trace elements in whole tissue of beef heart

Beef heart tissue was found to contain the same elements in similar concentrations as human heart tissue. The concentration of some elements, however differed significantly Table I shows a compilation of the results obtained from human whole heart tissue (II) and from whole heart tissue of beef heart (III and IV) Less Cd Co Se and Zn was found in the beef hearts than in the human hearts (significance***) A lower concentration of Co and Zn in beef heart tissue than in human heart tissue has previously been reported (49) The amount of Ag was also somewhat lower in the beef hearts than in the human hearts (significance*) whereas the amount of Ce was somewhat higher (significance*) The other 17 trace elements investigated did not differ significantly The beef heart material comprised samples from 2 calves and 7 adult animals. No obvious differences could be discerned between the trace element concentration in the samples from the calves and those from the adult animals Possibly the Hg values were somewhat lower in the former than in the latter The samples from the adult animals consisted partly of a mixture of muscle tissue from the anterior and posterior wall of the left ventricle and partly of muscle tissue from the ventricular septum No apparent differences were noted between the trace element concentrations in different parts of the left ventricle. The lowest concentration of Au and Cu was, however observed in the ventricular septum

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The different subcellular fractions investigated, i.e. nuclear and cell residue fraction mitochondrial fraction, sarcotubular fraction and supernatant were obtained by differential centrifugation in sucrose solution The homogeneity of

other hitherto unknown metalloenzymes.

Trace elements in the conducting system of beef heart tissue

Various chemical components have been studied in the conductive tissue of the heart, including the bulk elements Na and K (e.g 24 50 41—43 72) Semiquantitative studies have also been made of some trace elements in the conductive tissue (41) Quantitative determinations of trace elements in this tissue seem, however to be completely lacking The aim of the present work (IV) was therefore to determine the amounts of a great number of trace elements in the conductive tissue of beef heart, and to compare them with the amounts of the corresponding trace elements in adjacent muscle tissue.

The atrioventricular node (AV node) the bundle of His and adjacent atrial and ventricular muscle tissue from four cattle hearts were investigated w th respect to the concentration of the following trace elements Ag, As, Au, Ba, Br Ca, Cd Ce, Co Cr Cs, Cu, Fe Hg, La, Mo, Rb Sb, Sc, Se, Sm, W and Zn. The bulk elements K, N and P were also determined The amount was expressed in µg/g wet tissue Statistical calculations were limited to the bulk elements and those trace elements which were previously observed in normal distribution in human heart tissue (II)

Different patterns of distribution were observed in comparison between the amounts of the elements in the AV node, the bundle of His and adjacent atrial and ventricular muscle.

As far as the bulk elements are concerned, the ventricular septum was found to contain the largest amount of K and P and the smallest amount of Na. The bundle of His contained about half the amount of K and P present in the septum, whereas the Na content was enriched. These differences were significant. Significant differences were also present between the right atrium and the AV node with respect to the increased amount of Na and the reduced amount of P in the node, whereas no significant difference in K was noted. The right atrium contained significantly more Na and almost significantly less K and P than the ventricular septum. No significant difference was present between the various parts of the conducting system with respect to the concentration of K, Na and P

Among the trace elements with known biological function, Cu, F and Zn were found to be greatly reduced in the conductive tissue as compared to adjacent muscle. The concentration of Cu, Fe and Zn in the ventricular septum was found to be 3—4 times than in the bundle of His (significance* for Cu and Fe, for Zn) The reduction in the AV node compared to adjacent atrial muscle was significant for Cu and almost significant for F and Zn. Comparison between the ventricular septum and the right atrium showed an almost significantly smaller amount of Cu in the latter The amount of Cu and Zn in the AV node and in the bundle of His did not differ significantly The value for Fe was, however higher in the node than in the bundle, which may perhaps have been due to a higher content of blood in the former The

gical function showed some notable patterns of distribution. Ca and Cu were highly enriched in the mitochondrial fraction. The enrichment of Ca is consistent with the well documented ability of mitochondria to accumulate Ca (e.g. 27 54 81 95) No concomitant enrichment of P was observed. The enrichment of Cu is in agreement with the localization of cytochrome oxidase, a Cu enzyme present only in the mitochondria (18 25 85) Co was found to be somewhat enriched in both the mitochondrial and the sarcotubular fraction The Co enrichment in the mitochondrial fraction was *of the same order of magnitude as that* reported for vitamin B_{12} in mouse liver mitochondria (86) Fe showed an enormous enrichment in the sarcotubular fraction which — with great probability — was due mainly to the accumulation of ferritin particles in this fraction (cf. III fig 2) The concentration of Fe in the mitochondrial fraction was found to be somewhat higher than in whole heart tissue, which may be compared to the association with mitochondria of different Fe enzymes, e g succinic dehydrogenase (18 25 85) and various cytochromes (8 28 85) No large differences were present between the different subcellular fractions with respect to Zn concentration. On a protein basis, the amount of Zn was, however somewhat enriched in the supernatant and the mitochondrial fraction which may be compared to the reported localization of various Zn enzymes in liver tissue from different animals i.e alcohol dehydrogenase (25 28) and carbonic anhydrase (25) in supernatant and glutamic dehydrogenase in mitochondria (25)

Reproducible and characteristic patterns of distribution in the different subcellular fractions were also obtained for the trace elements with suspected and with no known biological function. Ba, Hg La, Sm and W were highly and Mo somewhat enriched in the mitochondrial and sarcotubular fractions, whereas Br Cs and Rb were reduced in these fractions On a protein basis, the highest concentration of As, Br Cs, Mo Rb and Se was found in the supernatant. The *high concentration of Mo in the supernatant* may be compared to the localization of the Mo-containing enzyme xanthine oxidase, *which in rat liver tissue is* stated to occur only in the supernatant (25 96)

As a rule, good parallelism was noted in comparisons between the distribution of known metalloenzymes reported in different tissues from various animals and the distribution of corresponding trace elements in subcellular fractions of beef heart tissue. Differences may however exist between different animals and organs as regards the distribution pattern of elements and metalloenzymes, respectively

The reproducible and characteristic patterns of distribution observed for the trace elements studied may indicate that, in addition to the trace elements with known biological function, further trace elements may be of biological significance Numerous enzymes are known to be localized in the mitochondria and the sarcotubular system. In this connexion it is interesting to observe the great enrichment of several trace elements in these fractions, and it seems reasonable to presume the existence of

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Trace elements in human myocardial infarction

The studies of trace element described in Parts 2 nd 3 were ll made on material from healthy individuals. Studies f trace element concentrations i diseased states may however be of consequence from several points of view Changes in concentration may reflect metabolic disturbances, and the result of determinations may contribute to a better understanding of the biochemistry of the disease Trace element imbalance may be the cause of some metabolic disturbances. Excess or deficiency of trace element may be an aetiological factor in the disease Moreover hanges in concentration may be of diagnostic alue

E idence of the importance of trace elements i fatty acid metabolism has been presented (21 61) and the possibil ty that trace lement may constitute n aetiological factor in coronary heart disease has lately attracted increasing interest (10 60 75, 76) A comparison between the trace lement concentration in therosclerotic heart tissue g un injured heart tissue from patient with myocardial infarction, and the concentration in normal heart tissue was therefore considered to be of value.

In recent years, considerable attention has been focused on the biochemistry of myocardial infarction. Some of the enzymic lterations occurring in patients with myocardial infarction have become of great diagnostic importance (e g 9) Changes in the concentration of some trace element in such patients hav also been reported both in blood (1 22 39 47 93 97 98) and in infarcted heart tissue (34 36, 57 91) A comparison between the trace element concentration in i jured and uninjured heart tissu from patient with myocardial infarction w s regarded a motivated for these reasons.

I jured and uninjured heart tissu from 12 utopsy cases w th myocardial infarction (8 men and 4 women) ranging in age from 58 to 86 years, was investig ted with respect t the concentration of the following trace element Ag, As, Au Ba, Br Ca, Cd, Ce Co Cr Cs, Cu F Hg La, Mo, Rb Sb Sc, Se Sm, Zn and W The bulk elements K Na and P were also determined.

The amou t was expressed in µg/g wet tissue The mean dry weight and nitrogen content were also given. The ge of the myocardial infarctions, estimated on both clinical and pathoanatomical data, ranged from 2 hours to 14 days. The degree of fibrosis of the heart tissue was evaluated microscopically

A comparison between the trace le ment concentration in µg/g wet tissue i normal heart tissue from utopsy of 20 victims of traumatic accidents (II) and the corresponding concentrations in un-

amount of Ca was — contrary to Cu Fe and Zn — found to be enriched in the conductive tissue whereas Co occurred in low concentration, especially in the bundle of His

Among the trace elements with suspected biological function Ba, Br and Cd were found to be somewhat enriched in the conductive tissue as compared to adjacent muscle tissue, whereas the reverse applied to Rb The right atrium contained twice as much Br as the ventricular septum. The distribution of Cr varied from heart to heart whereas no obvious differences were recorded between the different kinds of heart samples as regards Mo and Se concentration

Among the trace elements without known biological function Ag Au, Sb and Sc were found to be somewhat enriched and Cs and Hg somewhat reduced, in the conductive tissue as compared to adjacent muscle tissue

The enrichment of some elements and the reduction of others in the conductive tissue, as compared to the common myocardium, may reflect metabolic differences since, in general, trace elements are known to participate in enzymic processes.

The small amount of several trace elements, e g Cu and Fe, recorded in the conductive tissue can be compared to the small amount of cytoplasmic organelles known to be present in this tissue (66) A more detailed understanding of the possible role of the trace elements in the conductive tissue must, however await more basic data on the biochemistry of this tissue

A linear relation between the difference in concentration and the age of myocardial infarction was also obtained for Ca, P, La and Zn. With increasing age of the myocardial infarction, less P and Zn and more Ca and La were present in the injured tissue.

The possibility of trace metal imbalance in patients with arterial hypertension has been pointed out by Schroeder (74, 78). Five of the 12 autopsy cases with myocardial infarction had a history of hypertension. A comparison between the hypertensive and the normotensive group with respect to the trace element concentration in the uninjured tissue revealed an almost significantly reduced amount of Br, Co and Sc and a low concentration (but insignificantly decreased) of Fe, Mo and Se ($p = 0.07$, 0.07 and 0.06, respectively).

Attention was also paid to the administration of diuretics and/or digitalis. Six of the 12 autopsy cases with myocardial infarction had been treated with diuretics for 1 year or longer and 6 had been treated with digitalis for 1 year or longer. In the group treated with diuretics, which coincides with the hypertensive group except for one case, an almost significant decrease in Br and low amounts (but insignificantly decreased) of Ca and Fe ($p = 0.09$ and 0.07, respectively) were obtained in the uninjured heart tissue in comparison with those cases which had not been treated with diuretics for any lengthy period. Two of the 12 autopsy cases had been treated with mercurial diuretics. High amounts of Hg were observed in the heart tissue of these cases. Accumulation of Hg in the tissue of patients treated with mercurial agents has previously been reported (36). The trace element concentration in the uninjured heart tissue in the groups with and without digitalis therapy did not, however, differ significantly.

injured heart tissue from autopsy of the 12 cases with myocardial infarction revealed no significant differences for most of the trace elements Cu and Mo were, however somewhat lower in the group with myocardial infarction and As and Ce somewhat higher The difference in As is possibly to be ascribed to the different age distribution in the two materials. No significant differences with age could, however be detected in either material. A possible correlation between As and age seemed, however to be present in the normal material, which suggests that a significant correlation might be obtained in a larger material The difference in Mo in the two materials may perhaps be ascribed to a varying degree of fibrosis. The concentration of Mo in the uninjured tissue from the patients with myocardial infarction decreased with increasing grade of fibrosis.

A comparison between the trace element concentration in μg/g wet tissue in the injured and uninjured heart tissue from infarcted hearts disclosed considerable differences with respect to several elements It seems reasonable to presume that a damaged tissue such as infarcted heart tissue will release many of its constituents. This did in fact, apply to some elements. Other elements were, on the contrary found in raised concentration in the injured tissue

In agreement with other investigations, the infarcted tissue was found to have a low content of the bulk elements K and P and a high content of Na (29 45 46 58)

In the group of trace elements with known biological function, Co and Zn were present in decreased concentration in the infarcted tissue Ca, on the other hand, was — in agreement with the results of Meister and Schumann (57) — found to be highly enriched. The high Ca content is consistent with the observation that dystrophic calcification occurs in early infarcted myocardial tissue (100) which, as pointed out by Jennings and Wartman (46) is remarkable in view of the fact that calcification of injured tissue is usually considered to be a late event The reduction of Zn in the injured tissue is compatible with the finding that lactic dehydrogenase, a Zn enzyme, disappears from infarcted heart tissue (65) Histochemically Fe has been shown to be deposited in the infarcted myocardium of dogs (100) On the other hand myoglobin is stated to be reduced in infarcted human heart tissue as compared to unaffected areas of the same hearts (7 11) In the present study no significant difference was recorded between injured and uninjured tissue from infarcted hearts as regards Fe content.

Among the elements with suspected biological function, an increased amount of Ba and Br and a reduced amount of Rb were noted in the injured tissue as compared to the uninjured In the case of Mo the reduction in the injured tissue was not significant according to the sign test However when the difference between Mo concentration in injured and uninjured tissue was plotted against the age of the myocardial infarction, a linear relation was found

Among the trace elements without known biological function, an increased amount of Sb and the lanthanides Ce La and Sm and a reduced amount of Cs were obtained in the injured tissue

different animals and the distribution of corresponding trace elements in subcellular fractions of beef heart tissue.

12 Among the trace elements with known biological function studied in subcellular fractions, Ca and Cu are highly enriched in the mitochondrial fraction, and Fe in th sarcotubular fraction. Co shows a slight increase in both the mitochondrial and the sarcotubular fraction. On a protein basis, Zn is obtained in the highest concentration in the supernatant. Among the trace elements with suspected biological function and without known biological function, Ba, Hg La, Mo, Sm and W are obtained in high concentration in both the mitochondrial and the sarcotubular fraction, whereas these fractions have a reduced ontent of Br Cs and Rb compared to whole heart tissue On a protein basis, the highest concentration of As, Br Cs, Mo Rb and Se is found in the supernatant.

13 In the beef heart, Ag, As, Au, Ba Br Ca, Cd, Ce, Co Cr Cs, Cu, F Hg, La, Mo Rb Sb, Sc Se, Sm, W and Zn are determined quantitatively in different parts of the conducting system (AV node nd bundle of His) and n adjacent tissue (right trium and ventricular septum) The bulk elements K, Na and P are also determined

14 The concentration of Ca, Cu, Fe Hg K, P Rb and Zn in µg/g wet tissue is found to be lower in the conductive tissue than in adjacent heart tissue, whereas the everse pplies to Ag Au, Ba, Br Cs, Cd, Na, Sb and Sc

15 The concentration of Br Cs, Co Cs and F In µg/g wet tissue is somewhat higher in the AV node than in the bundle of His.

16. In the right atrium, the concentration in µg/g wet tissue of Cu, Fe K, P and Zn is lower than in the ventricular septum, and that of Br Ca and Na is higher

17 Injured and uninjured heart tissue from patients who died of myocardial infarction is investigated with respect to the concentration of the following trace elements Ag As, Au Ba, Br Ca, Cd Ce Co, Cr Cs, Cu, Fe Hg La Mo Rb Sb, Sc, Se Sm, W and Zn The bulk elements K, Na and P are also determined.

18 A comparison is made between the trace element concentration in µg/g wet weight in normal human heart tissue from autopsy cases of traumatic accidents and the corresponding concentration in uninjured heart tissue from autopsy cases with myocardial infarction It reveals no significant differences w th respect to most of the elements. The concentration of Cu and Mo is, however lower and the concentration of As and Ce higher in the uninjured tissue from infarcted hearts than in normal human heart tissu

19 The concentration of Mo in the uni jured tissue from infarcted hearts is correlated to the degree of myocardial fibrosi m the infarcted hearts. Thus, the concentration decreases with increasing degree of fibrosis

20 A comparison between the trace element concentration in µg/g wet tissue in the injured and uninjured tissue from infarcted hearts discloses a decreased content of Co Cs, P Rb and Zn and an increased content of Ba, Br Ca, Ce, La, W Sb and Sm in the injured tissue.

21 The differences between the concentration of Ca, La, Mo, P and Zn in injured and in uninjured tissue from

General summary

The results of the present investigations can be summarized as follows

1 A recently developed ion-exchange technique combined with subsequent γ spectrometry is found to be advantageous in the determination of a large number of trace elements in biological material

2 Recovery values of ≥ 90 % are obtained for most of the elements investigated and the standard error of the mean of all 25 trace elements studied is ≤ 3 %

3 The following trace elements are determined quantitatively in the left ventricle of human hearts from autopsy victims of traumatic accidents Ag As, Au, Ba, Br Ca, Cd, Ce Co Cr Cs, Cu Fe, Hg La Mo Pt, Rb Sb Sc, Se Sm W and Zn. Central values and range of concentration are given on a wet weight basis

4 All the trace elements studied are detected in every sample with the exception of Hg in one sample Most of the trace elements are found to have a skew distribution Only the distribution of Cu, Fe Se and Zn appears normal.

5 The trace elements with known biological function (Ca, Co Cu, Fe and Zn) show with the exception of Co the least degree of variation whereas most of those without known biological function (Ag, As, Au, Ce, Cs, Hg La Pt, Sb Sc, Sm and W) vary greatly particularly Au and Sm. The degree of variation of the trace elements with suspected biological function (Ba Br Cd, Cr Mo Rb and Se) is, in general something intermediate.

6 No obvious differences in the trace element concentration with age or sex are detected

7 The left ventricle of beef hearts is found to contain the same trace elements in similar concentrations as the left ventricle of human hearts A smaller amont of Ag Cd Co Se and Zn and a somewhat higher concentration of Ce are however found in beef heart tissue than in human heart tissue.

8 No differences are apparent between calves and adult animals with respect to trace element concentrations in the heart tissue.

9 No evident differences are found between the trace element concentration in different parts of the left ventricle of the beef heart

10 As, Br Ca, Co Cs, Fe, Hg, La Mo P Rb Se, Sm, W and Zn show reproducible and characteristic patterns of distribution in subcellular fractions of beef heart tissue (nuclear and cell residue fraction, mitochondrial fraction sarcotubular fraction and supernatant)

11 As a rule, good parallelism is noted in comparisons between the distribution of known metalloenzymes in subcellular fractions from various tissues of

different animals and the distribution of corresponding trace elements in subcellular fractions of beef heart tissue

12. Among the trace elements with known biological function studied in subcellular fractions Ca and Cu are highly enriched in the mitochondrial fraction, and Fe in the sarcotubular fraction. Co shows a slight increase in both the mitochondrial and the sarcotubular fraction. On a protein basis, Zn is obtained in the highest concentration in the supernatant. Among the trace elements with suspected biological function and without known biological function, Ba, Hg, La, Mo, Sm and W are obtained in high concentration in both the mitochondrial and the sarcotubular fraction, whereas these fractions have a reduced content of Br, Cs and Rb compared to whole heart tissue. On a protein basis, the highest concentration of As, Br, Cs, Mo, Rb and Se is found in the supernatant.

13. In the beef heart, Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mo, Rb, Sb, Sc, Se, Sm, W and Zn are determined quantitatively in different parts of the conducting system (AV node and bundle of His) and in adjacent tissue (right atrium and ventricular septum). The bulk elements K, Na and P are also determined.

14. The concentration of Cs, Cu, Fe, Hg, K, P, Rb and Zn in µg/g wet tissue is found to be lower in the conductive tissue than in adjacent heart tissue, whereas the reverse applies to Ag, Au, Ba, Br, Ca, Cd, Na, Sb and Sc.

15. The concentration of Br, Ca, Co, Cs and Fe in µg/g wet tissue is somewhat higher in the AV node than in the bundle of His.

16. In the right atrium, the concentration in µg/g wet tissue of Cu, Fe, K, P and Zn is lower than in the ventricular septum, and that of Br, Ca and Na is higher.

17. Injured and uninjured heart tissue from patients who died of myocardial infarction is investigated with respect to the concentration of the following trace elements: Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mo, Rb, Sb, Sc, Se, Sm, W and Zn. The bulk elements K, Na and P are also determined.

18. A comparison is made between the trace element concentration in µg/g wet weight in normal human heart tissue from autopsy cases of traumatic accidents and the corresponding concentration in uninjured heart tissue from autopsy cases with myocardial infarction. It reveals no significant differences with respect to most of the elements. The concentration of Cu and Mo is, however, lower and the concentration of As and Ce higher in the uninjured tissue from infarcted hearts than in normal human heart tissue.

19. The concentration of Mo in the uninjured tissue from infarcted hearts is correlated to the degree of myocardial fibrosis in the infarcted hearts. Thus, the concentration decreases with increasing degree of fibrosis.

20. A comparison between the trace element concentration in µg/g wet tissue in the injured and uninjured tissue from infarcted hearts discloses a decreased content of Co, Cs, P, Rb and Zn and an increased content of Ba, Br, Ca, Ce, La, Na, Sb and Sm in the injured tissue.

21. The differences between the concentration of Ca, La, Mo, P and Zn in injured and in uninjured tissue from

infarcted hearts are correlated to the age of the myocardial infarction. With increasing age of the infarction Ca and La increase, and Mo P and Zn decrease

22 A comparison is made between the trace element concentration in $\mu g/g$ wet tissue in the uninjured tissue of infarcted hearts of patients with and without a history of arterial hypertension In the former cases, the concentration of Br Co and Sc is almost significantly reduced and that of Fe, Mo and Se is low but insignificantly decreased

23 A comparison is also made between the trace element concentration in $\mu g/g$ wet tissue in the uninjured tissue of infarcted hearts of patients with and without a history of treatment with diuretics. In the treated cases, the Br concentration is almost significantly decreased and that of Ca and Fe is low although the decrease is not significant.

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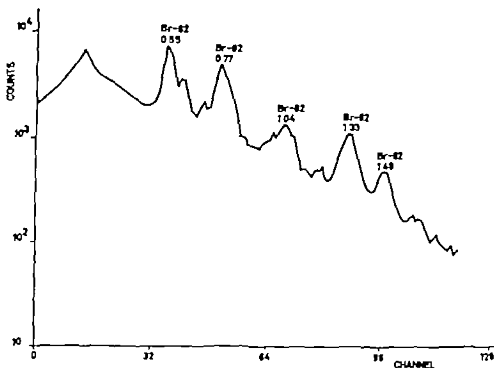
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Appendix

Diagrams 1—14 show typical γ-spectrograms obtained from irradiated and separated human heart samples. They derive from the 14 different ion-exchange resins or precipitations mentioned on page 14 which are obtained in the chemical group separation system used (fig 1) Recording was made with an Autograph recorder (F L. Mosely Co model 2D-2) In diagrams 1 6, 7 and 9 counting was performed with the sample placed close to the 3" × 3" well-type crystal, whereas in the other diagrams the sample was placed inside the well of the crystal.

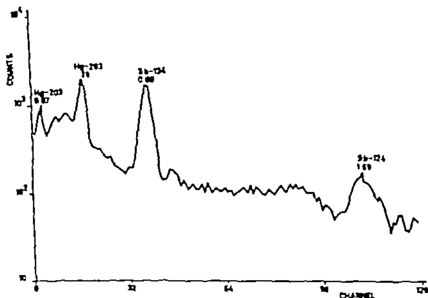
With the group separation system used, highly complex γ-spectrograms are not, for the most part, obtained Thus, all except four diagrams (nos 5 6 11 and 14) belong to one or two nuclides. The evaluation of some complex spectra is facilitated by repeated counting after suitable cooling periods. Thus, after the activities of Cu^{64} La^{140} and Sm^{153} recorded in diagram 11 have been allowed to decay the amount of Ce and Co is easily calculated. In diagram 13 the activity of Na was allowed to decay before recording the activity of Cs^{134} and Rb^{86}

DIAGRAM 1 Alkali absorber



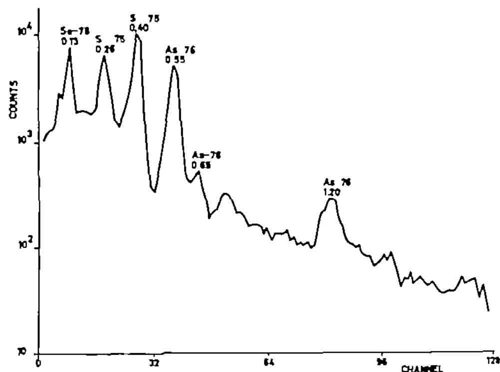
Irradiation 1.83 $10^{[illegible]}$ n/cm sec for 48 h
Cooling period 5 days
Counting time 2 min
Sample close to the crystal

DIAGRAM 2. Anion exchanger Cl form I



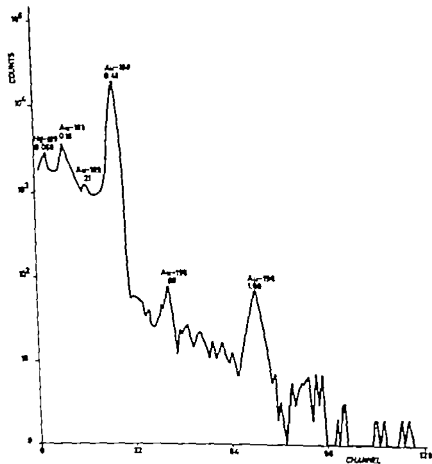
Irradiation 1.91 $10^{[illegible]}$ n/cm² sec for [illegible] h
Cooling period 25 days
Counting time 10 min
Sample inside the well of the crystal

Diagram 3 Precipitate As, Se



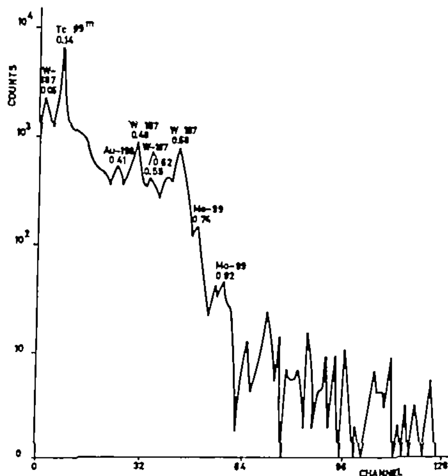
Irradiation 1.84 10^{14} /cm sec for 48 h
Cooling period 5 days
Counting time 10 min
Sample inside the well of the crystal

DIAGRAM 4 Anion exchanger Cl form II A



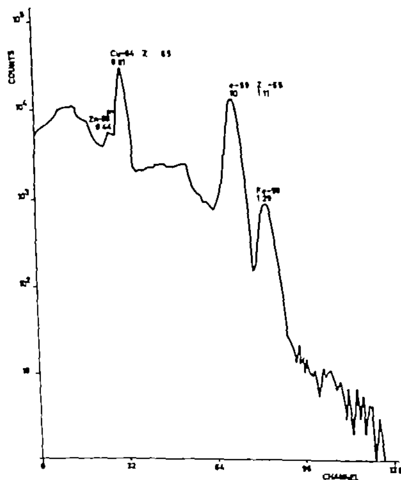
Irradiation 2.00 10[illegible] n/cm sec for 48 h
Cooling period 5 days
Counting time 2 min
Example inside the cell of the crystal

DIAGRAM 5 Anion exchanger Cl form II



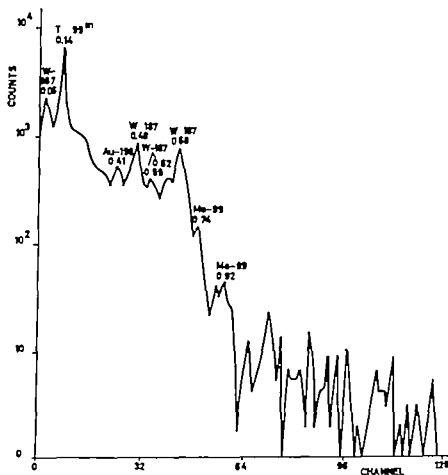
Irradiation 2.00 10[?] n/cm sec for 48 h
Cooling period 5 days
Counting time 40 min
Sample inside the well of the crystal

Diagram 6. Cation exchanger H form III



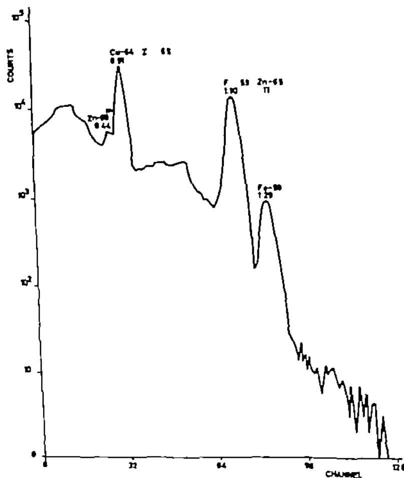
Irradiation 2.00 10[illegible] cm² sec for 48 h
Cooling period 3 days
Counting time 10 min
Sample close to the crystal

DIAGRAM 5. Anion exchanger Cl form II



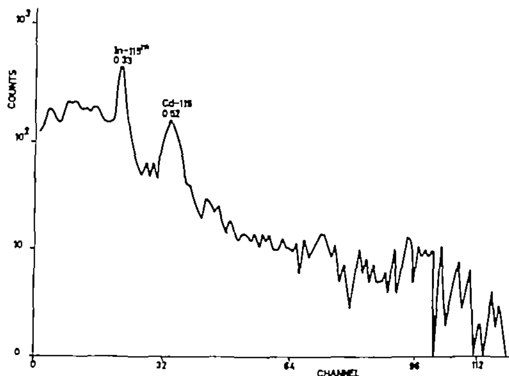
Irradiation 2.00 10^{12} n/cm sec for 48 h
Cooling period 5 days
Counting time 40 mm
Sample inside the well of the crystal

Diagram 6. Cation exchanger H form III



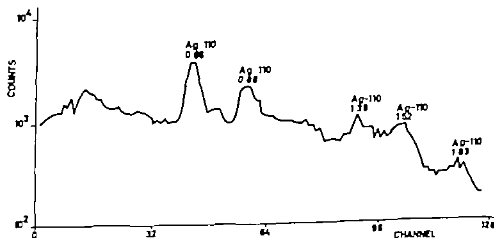
Irradiation 2.00 10[illegible] n/cm² sec for 48 h
Cooling period 3 days
Counting time 10 min
Sample close to the crystal

DIAGRAM 7 Cation exchanger H form IV



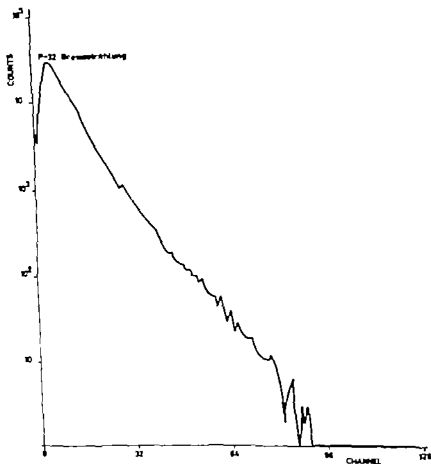
Irradiation 1.91 10^{13} n/cm sec for 65 h
Cooling period 8 days
Counting time 10 min
Sample close to the crystal

DIAGRAM 8. Anion exchanger Cl form IV A



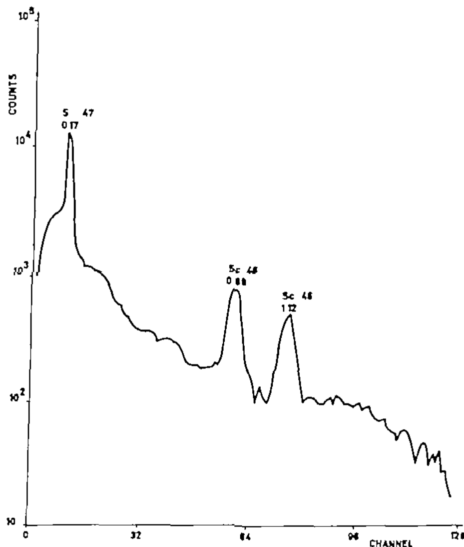
Irradiation 1.83 10^{13} n cm sec for 24 h
Cooling period 270 days
Counting tim 400 min
Sample inside the w ll of the crystal

DIAGRAM 9. Phosphate precipitate



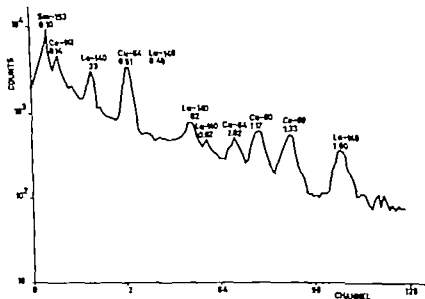
Irradiation 1.83 · 10^{14} cm sec for 65 h
Cooling period 42 days
Counting time 1 min
Sample close to the crystal

DIAGRAM 10 Anion exchanger OH form V



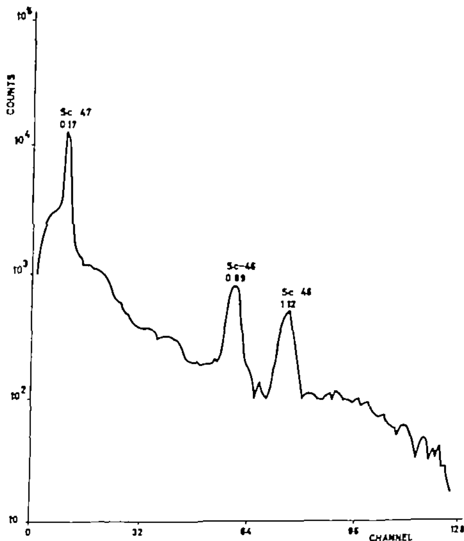
Irradiation 1.56 10[13] n/cm sec for 65 h
Cooling period 8 days
Counting time 40 min
ample inside the well of the crystal

DIAGRAM 11 Anion exchanger Citrate form VI



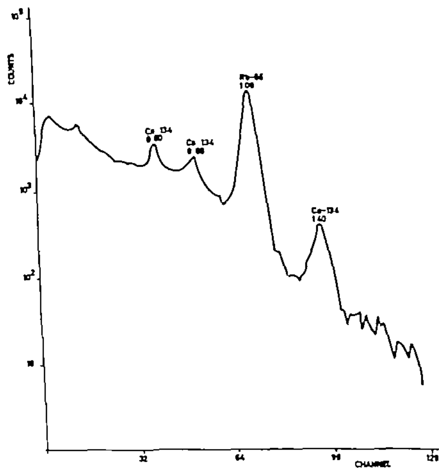
Irradiation 1.81 10^{12} n/cm² sec for 63 h
Cooling period 6 days
Counting time 10 min
Sample inside the well of the crystal

DIAGRAM 10. Anion exchanger OH form V



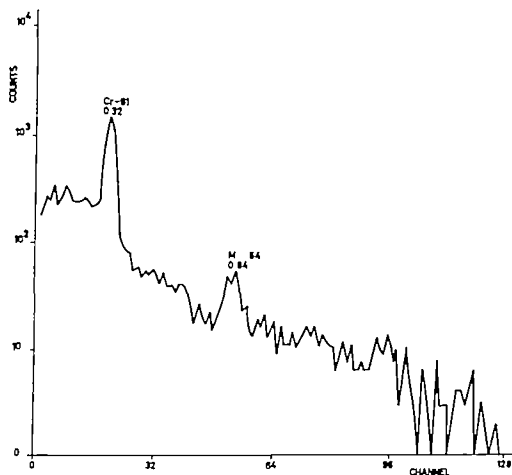
Irradiation 1.56 10[illegible] n/cm sec for 65 h
Cooling period 8 days
Counting time 40 min
ample inside the w ll of the crystal

DIAGRAM 13. Cation exchanger Na form VIII



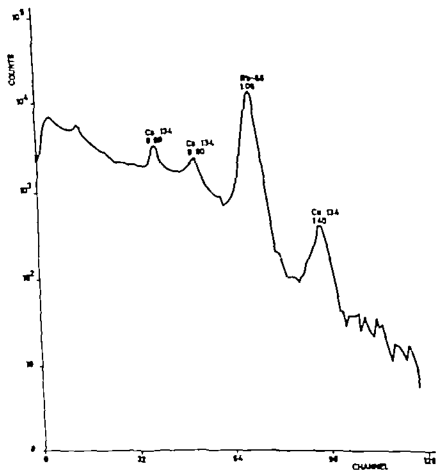
Irradiation 2.00 10[illegible] n/cm sec for 48 h
Cooling period 18 days
Counting time 20 min
Sample inside the well of the crystal

Diagram 12. Anion exchanger OH form VII



Irradiation 2.00 10¹³ n/cm sec for 48 h
Cooling period 7 days
Counting time 10 min
Sample in the well of the crystal

DIAGRAM 13. Cation exchanger Na form VIII



Irradiation 2.00 10[illegible] n/cm^2 sec for 48 h
Cooling period 18 days
Counting time 20 min
Sample inside the well of the crystal

STOCKHOLM 19[illegible]

KUNGL. BOKTRYCKERIET P. A. NORSTEDT & SÖNER

ACTA MEDICA
SCANDINAVICA

SUPPLEMENTUM 438

LONG-TERM ANTICOAGULANT THERAPY IN PATIENTS WITH CEREBRAL INFARCTION

A controlled clinical study

BY

ERIK ENGER and SVEIN BØYESEN

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Long-Term Anticoagulant Therapy in Patients with Cerebral Infarction

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Contents

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Introduction

Brain damage caused by atherosclerotic lesions of the brain arteries is responsible for about 15 per cent of the total number of deaths in the Western European countries. In addition the survivors of a stroke represent kind of suffering and restricted activity of life which cannot be over-estimated.

This study is concerned with the effect of long-term peroral anticoagulant therapy in patients who have survived a cerebral damage caused by an occlusive arterial process presumably of a thrombotic or atheromatous nature. The role of anticoagulants in the treatment of patients with such lesions both of cerebral and coronary arteries has been one of the most debated questions in clinical medicine during the past 15 years. When this therapy was introduced, too little clinical and pathological information was available on the natural course of diseases related to atheromatous lesions. In addition the initial studies on anticoagulants failed to fulfil some of the basic requirements of clinical trials, above all that of using adequate controls. Reports are still appearing in which requirements of a controlled clinical trial are not fulfilled. The important principle of unity in place and time is frequently overlooked. During the allocation procedure one should use constructed random list or a central card system. The referring doctor should be without influence on or knowledge of the distribution within the groups. It may also be advisable to make subadjustments with respect to important factors, for instance age and sex. Except for the therapy to be tested both series should be given the same medical care and supervision. This implies the use of placebo therapy and the double blind technique. During a study of anticoagulants the essential finding is that of thrombo-embolic manifestations. However the clinical and even the pathological criteria of such phenomena are far from clear-cut. The diagnosis is frequently dependent on poorly defined symptoms and signs. It may be important that the clinicians and the pathologists are uninfluenced by the knowledge of the treatment scheme. The aim of anticoagulant therapy is to induce a hypocoagulability of blood which might have a favourable influence on thrombotic processes. Mortality figures have their meaning only when related to thrombo-embolic or hemorrhagic processes revealed at autopsy.

In the presentation three questions should be given particular attention. What was the method of blood control? What level of hypocoagulability was intended? What kind of anticoagulant effect was achieved? The last point is frequently omitted. The statement that a reduction between 10 to 30 per cent of normal of the prothrombin level was intended is without value unless the actual results are presented. If these are omitted one is left with uncertainty as to the real difference between the treated and control series. A report on thrombo-embolic phenomena carries weight only if associated with the documentation that the patients were under

the original plan. In the present study Owren's thrombotest method was used for blood control. In the majority of Anglo-American studies other methods have been used. These are usually less sensitive and involve a greater risk of hemorrhagic complications. This was the main reason why we continued our study instead of concluding earlier than intended owing to the results of other workers.

optimal anticoagulant therapy during the relevant period.

Some statistical aspects of clinical trials deserve attention. Unless the study is followed by the sequential analysis technique the number of patients and the length of the observation period should be decided beforehand. One has sometimes the feeling that these decisions may have been influenced by casual factors. In biological studies a difference at the 5 per cent level of probability is usually accepted as being of statistical significance and hence of real value. But a difference at the 10 per cent level is not necessarily without clinical importance. Groups of human beings can never be constructed in a way which completely prevents some degree of heterogeneity. This implies that the general results of the total series of patients are not always applicable to subgroups, for instance in the two sexes or in different age groups. Especially when the results show a definite trend is it important that the material should be sufficiently large to permit a statistical analysis within subgroups. The significance of including a very large number of patients in a clinical trial may be debated. It is important to include a sufficient number of patients to permit an adequate statistical analysis. However, the benefit of a therapeutic regime is most evident if only a moderate number of cases are required to prove its effect. If exceptionally large case materials are necessary to prove the value of a treatment, it may well be that practical and economic reasons will militate against its adoption in clinical practice.

During recent years many attempts have been made to establish a possible benefit from peroral anticoagulants in cerebrovascular disease. The need for a large number of cases became evident at an early stage. This gave rise to co-operative studies involving several medical centres and a great number of doctors and technicians introducing an uncertainty with respect to the clinical follow-up and blood control. Although patients at different centres are selected, randomized, and followed according to well defined plans, they might well be submitted to a different medical care and supervision related to geographical and personal factors. The variability thus introduced and the failure of personal responsibility for the individual patient may be illustrated by the fact that in one of the largest studies in this field 60 per cent of the cases in the treated series were lost during the observation period (Baker et al. 1962). Clinical studies are best performed with as few doctors as possible. The anticoagulant studies by Bjerkelund (1957) and Borchgrevink (1960) are good examples.

When the present study started 5 years ago only few papers concerning the long term effect of anticoagulants in cerebrovascular disease had appeared. None of them were adequately performed. We started with the hope of adding information to this field by adopting an adequate technique which might counterbalance the restricted number at a single hospital of cerebrovascular patients suitable for long-term study on an out-patient basis. During these years several well-conducted studies have been presented. While the early papers in this field were in favour of the long-term use of anticoagulants, the recent ones usually give evidence against its value. Since most of the latter studies have included control series the problem has apparently been solved. We were on the point of deciding to conclude the study earlier than intended when the paper of Bradford Hill, Marshall & Shaw (1960) appeared. Not only were these authors unable to demonstrate any effect from anticoagulants but the mortality of their treated patients was greater than that of the control patients owing to an increased frequency of cerebral hemorrhage. Their study was concluded earlier than intended because it was found unethical to continue according to

the brain is a thrombotic occlusion necessary for the development of a necrotic area of clinical significance.

From such considerations it may be concluded that the possible benefit of anticoagulants in the long-term treatment of coronary artery disease by no means warrants conclusions as to their value in cerebral infarction.

In the great majority of patients with cerebrovascular disease an ischemic necrosis or hemorrhage is responsible for the clinical manifestations. When concerned with the use of anticoagulants the distinction between these conditions is of paramount importance. The varied pathogenetic mechanisms which may be responsible for the cerebral infarction should also be recalled. Arterial spasms, thrombotic occlusions, embolic occlusions derived from atheromatous plaques in the great vessels of the neck or from intracardiac thrombi, and circulatory insufficiency due to reduction of the blood flow to vessels narrowed but not occluded by atheroma, have all been advanced as possible causes of cerebral infarction. Probably all these mechanisms may be of clinical importance. But the evaluation of their relative frequency is difficult. It is reasonable to assume that cerebrovascular symptoms are frequently produced by the combination of structural changes in cerebral vessels and general cardiovascular disturbances. The cerebral circulation should be considered a function of the total cardiovascular system. Circulatory changes related to decreased cardiac output can only be recognized in the living patient and may be the reason why an occluding vessel is not found at autopsy despite the presence of a cerebral infarction. Another reason why the pathologist may fail to demonstrate an occluded or narrowed vessel is that common sites of stenotic changes of the cerebral vessels are infrequently examined at autopsy because they are partly inaccessible. This applies mainly to the extracranial portion of the vertebral arteries. When the cerebral oxygen delivery is already compromised by atherosclerotic narrowing of the extracranial or intracranial vessels, it is easy to realize that a further reduction in blood flow of various origin, for instance by a myocardial infarction, cardiac arrhythmias, the influence of hypotensive drugs, systemic hemorrhages etc., may additionally impair oxygenation of cerebral tissue, so that ischemic dysfunction or even an infarction may occur. The variability of intracranial arteries can easily be demonstrated by angiography or by operation (Emblem 1961). It is difficult, however to decide to what extent constrictive mechanisms may be responsible for irreversible ischemic damage. If the cerebral circulation has been previously compromised, additional arterial spasms may be of importance. Finally the frequent co-existence of intracranial and extracranial arterial lesions should be recalled.

Before the patient with a cerebrovascular accident is included in a clinical study every effort should be made to elucidate the pathogenetic mechanisms involved. This will increase the homogeneity of the case material. Apart from a general medical and neurological examination cerebral angiography is particularly useful. The method has certain limitations. Extracerebral arterial lesions can be detected with considerable accuracy but the intracerebral ones are less readily revealed. It is not an infrequent experience that this method fails to support even the obvious diagnosis of an intracranial cerebral vascular disease. The method carries some risk, but when a correct diagnosis is essential cerebral angiography should be performed. After the introduction of this method the high incidence of pathological changes of the carotid and vertebral arteries in the neck and at the base of the skull was discovered.

The syndrome of cerebral vascular insufficiency or transient attacks of ischemia

CHAPTER 1

Anticoagulants in Cerebrovascular Disease

Pathogenetic and terminological aspects

The long-term use of anticoagulants in the treatment of patients with cerebral infarction was initiated by the numerous reports of the beneficial effect of these agents in coronary artery disease. There are similarities between the two conditions. Atheromatous arterial intima lesions seem to be the primary factor in both. The macroscopical and microscopical resemblance between atherosclerotic lesions in cerebral and coronary vessels, as well as their frequent co-existence, suggest that the etiology of atherosclerosis is the same. Hypertension is commonly associated with the accelerated appearance of clinically significant arteriosclerotic degeneration in both organs.

When atheromatous intima lesions reach a sufficient size they may produce clinical symptoms by reducing the blood flow. They may also rupture into the lumen or give rise to intimal hemorrhage with the subsequent obturation of the vessel. However of the foremost importance in the development of a definite ischemic area is the irreversible platelet aggregation at the site of the atheromatous intima lesion, leading to an occluding thrombotic process. Above the age of 40 widespread atheromatous lesions in several parts of the body are common autopsy findings, but the great tendency of thrombotic occlusions to occur at atheromatous intima lesions most often in the coronary and in the cerebral vessels, remains unexplained.

Although the development of a myocardial and cerebral infarction may have common pathogenetic mechanisms, their clinical appearance differs considerably. In unselected case materials the age and sex distribution of patients with cerebral and myocardial infarction is different, the latter condition striking younger individuals and males more frequently than females. In patients with myocardial infarction the serum lipid pattern deviates more from normal than in patients with cerebral infarction, while the latter group shows a higher frequency of hypertensive individuals. These differences cannot be readily explained, but the pronounced circulatory discrepancy between the two organs should be recalled. In the heart muscle there are only poor possibilities for the development of collateral circulation. On the other hand, probably no other organ in the body is so well provided with collaterals as the brain. This is effected through the circle of Willis, through the meningeal vessels, and by the communications between the internal and external carotid arteries. Finally it should be emphasized that neither in the heart nor in

exclude in this study. This study may be said to deal with *non-embolic ischemic cerebral infarction* i.e. an ischemic condition of the brain producing persistent neurological defects.

Anticoagulant aspects

Neither heparin nor peroral anticoagulants can dissolve a fibrin clot. The effect to be expected from anticoagulant therapy is the prevention or limitation of a thrombotic process. If an ischemic cerebral infarction with tissue necrosis has occurred, restoration of blood flow to the damaged area will not restore function. An established cerebral infarction would not be changed by anticoagulants unless one postulates that collateral channels are protected by their effect. The following points illustrate the potentially beneficial mechanisms of anticoagulants once a cerebral infarction has developed:

a) Anticoagulants may hamper the propagation of the clot from the original thrombus distally or centrally. The spread of the thrombotic process often involves anastomotic vessels and anticoagulants may keep the infarcted area from enlarging. This effect is mainly related to the acute phase of the cerebrovascular accident.

b) Anticoagulants may prevent distal embolism from large thrombosed vessels such as the carotid or vertebral arteries into small peripheral vessels. An effect on distal embolism may also be exerted by influence on small thrombi or platelet clumps which are striking features of experimental cerebral thrombosis (Meyer 1958).

c) If the cerebral infarction is caused by atheromatous narrowing with or without thrombus, anticoagulant therapy may prevent the development of an occluding thrombotic process at this site or at other co-existing atheromatous lesions in the cerebral vessels. *This is the main purpose of long-term anticoagulant therapy in patients with an established cerebral infarction.*

d) Anticoagulants may prevent the development of venous thrombosis and subsequent pulmonary embolism. This effect is of particular significance in patients with a recent cerebrovascular accident. But in the long-term treatment of old and often disabled patients an effect on the venous side of the circulation may also be of importance.

e) There is experimental evidence to indicate that anticoagulants might increase the natural tendency to recanalization of an occluding thrombus (Wright *et al.*, 1953).

f) Still the general opinion is that atherosclerosis begins as degenerative change with deposition of cholesterol in the deep layers of the intima followed by fibrous tissue proliferation. However Duguid (1955) and Duguid & Robertson (1957) have advanced the theory based on experimental evidence that atheroma may be the result of a primary deposition of platelets and fibrin on the inner surface of the arteries which undergo secondary atheromatous and fibrous degeneration. Thus primary thrombogenic intimal changes may ultimately form site for secondary thrombotic deposition. Consequently long-term anticoagulant therapy may be of significance in preventing further development of atherosclerosis as well as the secondary thrombotic process at an atherosclerotic plaque already present.

Three dangers ar involved in the use of anticoagulants in cerebrovascular disease. There is the danger of giving anticoagulants in case of intracranial hemorrhage owing to diagnostic errors. There is the risk of inducing bleeding into and around an infarcted area. Finally a major visceral hem-

(T.I.A.) will not be given particular attention in this study but the term is repeatedly mentioned. The syndrome is of interest since it has been maintained frequently that anticoagulants are of value in preventing such attacks. The T.I.A. may be defined as *any single or repeated abrupt change in the neurological status due to local cerebral ischemia which subsides within a short time with no significant neurological deficit.* If there is a localized narrowing of a cerebral artery hemodynamic disturbances may cause *transient cerebral ischemia with temporary neurological deficit.* Such disturbances may be caused by a reduction in the *systemic blood pressure and the cardiac output*, and are influenced by the degree of patency of blood vessels of the neck. Several other conditions *may precipitate* a T.I.A. arterial spasms, cerebral thrombosis, small emboli hemorrhage, and brain tumour But in the case of repeated T.I.A.s the pathogenetic considerations will usually be limited to the above mentioned extracranial factors, to vasospasm, and to repeated small emboli.

The relative frequency of the different pathogenetic mechanisms involved in the development of the non-hemorrhagic cerebrovascular accident has been subject to considerable disagreement between *clinicians* and pathologists. This is mainly caused by their different approach. Hicks & Warren (1951) *failed to reveal an occlusive vascular* process in 60 per cent of patients with a large cerebral infarction. However this was a *retrospective* study and the examination of the extracranial portion of the cerebral arteries was not a routine procedure. In a prospective study the patterns of cerebrovascular diseases in Boston and Oslo were *compared (Torvik Jörgensen & Adams* 1965) No important divergences between the two cities were found. In patients dying of a *massive recent* cerebral infarction, they were able to demonstrate an occluding vascular process of thrombo-embolic nature in 89 per cent. There was an equal distribution of clots *presumed to* be thrombi and emboli. Their results furnish a good argument for the use of anticoagulants. Concerning autopsy findings *it should be realized* that even small cerebral *infarctions*, for instance with a diameter from 1/ to 3 cm, may be of clinical importance (Jörgensen & Torvik 1965) In lesions of that magnitude it is often impossible to demonstrate thrombo-embolic processes. Angiography is also non-contributory Finally there is the possibility *that thrombo-embolic* clots may be reabsorbed and recanalized during weeks or months after the cerebral accident. Thus it is *necessary to take the time factor into account* when autopsy findings are interpreted.

These are the pitfalls when the pathogenetic *mechanisms* of the *cerebrovascular* accident are to be reconstructed in the individual patient. The great variability of these mechanisms makes the evaluation of any treatment in cerebrovascular disease difficult and points to the importance of a complete documentation of the case material in such studies. In regard to terminology confusion *has arisen from the indiscriminate* use of the term cerebral thrombosis as a synonym for cerebral infarction. Cerebral *Infarction* is a well defined pathological term indicating the ischemic result of occluding cerebrovascular disease. The term cerebral thrombosis should be avoided with the possible exception of cases in which the presence of an intracranial vascular occlusion can be demonstrated by angiography Otherwise the term cerebral infarction or ischemic cerebral *infarction is preferable* These expressions also have the advantage of being equally suitable whether the atheromatous or stenotic lesion is intracranial or extracranial. The general term cerebral infarction seems to be most correct *and will be* used in the present study It has the disadvantage of including cases of cerebrovascular *embolism*, which we have tried to

exclude in this study. This study may be said to deal with *non-embolic ischemic cerebral infarction*, i.e. an ischemic condition of the brain producing persistent neurological defects.

Anticoagulant aspects

Neither heparin nor peroral anticoagulants can dissolve a fibrin clot. The effect to be expected from anticoagulant therapy is the prevention or limitation of a thrombotic process. If an ischemic cerebral infarction with tissue necrosis has occurred, restoration of blood flow to the damaged area will not restore function. An established cerebral infarction would not be changed by anticoagulants unless one postulates that collateral channels are protected by their effect. The following points illustrate the potentially beneficial mechanisms of anticoagulants once a cerebral infarction has developed.

a) Anticoagulants may hamper the propagation of the clot from the original thrombus distally or centrally. The spread of the thrombotic process often involves anastomotic vessels and anticoagulants may keep the infarcted area from enlarging. This effect is mainly related to the acute phase of the cerebrovascular accident.

b) Anticoagulants may prevent distal embolism from large thrombosed vessels such as the carotid or vertebral arteries into small peripheral vessels. An effect on distal embolism may also be exerted by influence on small thrombi or platelet clumps, which are striking features of experimental cerebral thrombosis (Meyer 1958).

c) If the cerebral infarction is caused by atheromatous narrowing with or without thrombus, anticoagulant therapy may prevent the development of an occluding thrombotic process at this site or at other co-existing atheromatous lesions in the cerebral vessels. *This is the main purpose of long-term anticoagulant therapy in patients with an established cerebral infarction.*

d) Anticoagulants may prevent the development of venous thrombosis and subsequent pulmonary embolism. This effect is of particular significance in patients with a recent cerebrovascular accident. But in the long-term treatment of old and often disabled patients an effect on the venous side of the circulation may also be of importance.

e) There is experimental evidence to indicate that anticoagulants might increase the natural tendency to recanalization of an occluding thrombus (Wright *et al.*, 1953).

f) Still the general opinion is that atherosclerosis begins as a degenerative change with deposition of cholesterol in the deep layers of the intima followed by fibrous tissue proliferation. However Duguid (1955) and Duguid & Robertson (1957) have advanced the theory based on experimental evidence that atheroma may be the result of a primary deposition of platelets and fibrin on the inner surface of the arteries which undergo secondary atheromatous and fibrous degeneration. Thus primary thrombogenic intimal changes may ultimately form a site for secondary thrombotic deposition. Consequently long-term anticoagulant therapy may be of significance in preventing further development of atherosclerosis as well as the secondary thrombotic process at an atherosclerotic plaque already present.

Three dangers are involved in the use of anticoagulants in cerebrovascular disease. There is the danger of giving anticoagulants in case of intracranial hemorrhage owing to diagnostic errors. There is the risk of inducing bleeding into and around an infarcted area. Finally major visceral hem-

orrhage may be produced followed by hypotension and further deterioration of the cerebral circulation.

The two first points deserve particular attention. The clinical distinction between an intracerebral hemorrhage and a cerebral infarction may be very difficult. Both conditions are frequently associated with arterial hypertension, and although the opposite course is the rule, the clinical development of a hemorrhage may be insidious, and that of an infarction may be abrupt. Hence every diagnostic effort should be made before patients with cerebrovascular diseases are given anticoagulants. The slightest suspicion *of a bleeding is an absolute* contraindication to anticoagulant therapy However mistakes *in diagnosis have to be accepted as* inevitable.

It is a well-established *fact that a cerebral* infarction may be associated with hemorrhage into the necrotic area apart from any influence by anticoagulants. In an autopsy study Jörgensen & Torvik (1965) have shown that this is a relatively frequent phenomenon. However in the majority of their cases the hemorrhagic infarctions were presumably occurring on an embolic and not on a thrombotic base. Furthermore, there is no information in their study on the possible influence of anticoagulants.

These facts emphasize the need for a precise *diagnosis. In the present study all* patients were submitted to a lumbar puncture, often repeatedly and in the majority of cases a cerebral angiography was made. *Even a trace of yellow colour* of the cerebrospinal fluid caused exclusion from the study The same applies to the slightest suspicion by angiography of an intracerebral expansion. Sometimes a cerebral infarction is associated with xanthochromic cerebrospinal fluid and may also show signs of an intracerebral expansion by angiography owing to edema. These facts did not influence our policy for the exclusion of cases.

The effect of *anticoagulants on an established* ischemic infarction is not entirely predictable. Theoretically there is a *definite* risk of inducing bleeding into and around the necrotic area. This is supported by experimental evidence in animals (Wood *et al.* 1958 Peterman *et al.* 1959) On the other hand, Sibley *et al.* (1957) were unable to observe such an effect of anticoagulants on ischemic infarcts in dogs. Furthermore, one should be very cautious of applying the results from animal experiments on cerebrovascular accidents in humans. Apart from the results of Bradford Hill *et al* (1960) clinical experience speaks against the assumption that anticoagulants involve a significant risk of inducing hemorrhage into a cerebral infarction (*Wright & McDevitt 1954 Fisher* 1958)

Coagulation of blood is a complex process in which a large number of factors play a part. The peroral anticoagulants suitable for long-term treatment, the coumarins and the indanediones, exert their effect by inhibiting the hepatic production of factor VI (prothrombin) factor VII (proconvertin) factor IX (antihemophilic factor B) and factor X (the Stuart Prower factor) None of the peroral anticoagulants are definitely superior to the others. In Norway phenylindanedione is the drug most commonly used. It has proved to be a stable and reliable *agent, the effect of which is easy to control* by suitable methods (Borchgrevink 1960 *Enger et al. 1963*)

There are three different methods for the control of blood hypocoagulability induced by this therapy In Anglo-American countries the estimation of the prothrombin time in seconds by the Quick one-stage procedure is most commonly used. The method is simple but has the disadvantage that the results are dependent on the potency of the tissue *thromboplastin added.* Therefore it is difficult to make a satisfactory standardization of the method. The more advanced and

reliable procedures of control, i.e. the P & P and the thrombotest methods, were both developed in this country. During the past 10 years these methods have been extensively used in the Scandinavian countries for control of anticoagulant therapy and have been proved extremely reliable. The prothrombin and proconvertin test (P & P method, Owren & Aas 1951) is more expensive and cumbersome than the Quick procedure. But this is more than outweighed by the more complete determination of the coagulation defects produced. Thus the method furnishes base for safe anticoagulant therapy. The introduction of the thrombotest (T. T.) method (Owren 1959) was a further advance. Its sensitivity to the depression of the various coagulation factors induced by anticoagulants makes it superior to the P & P method. In addition it can be performed on capillary blood. These 3 tests do not measure exactly the same thing. A result obtained by one procedure cannot be transformed into certain value within one of the other systems. If tests are performed simultaneously one might find that reading of two to two-and-a-half of the control time by the Quick method is equivalent to about 8 to 10 per cent by the P & P method and even lower by the thrombotest method (Keyser 1963).

In the present study as in most others in this field, patients with extremely high diastolic blood pressure values were excluded. Nevertheless, a series of patients with cerebrovascular disorders will include a considerable number of hypertensive individuals in whom there is an increased risk of provoking hemorrhage by anticoagulants. Furthermore, such study predominantly involves old people. Although patients suitable for long-term treatment are selected, the material will comprise a number of disabled patients with mental deterioration and reduced ability to co-operate. Co-operation is essential for the safe performance of anticoagulant treatment. Every effort should be made to establish a good doctor-patient relationship.

There is remarkably little experimental evidence on the optimal intensity of anticoagulant therapy. The therapeutic range is a poorly defined term, which rests mainly on an empiric base. Initially a P & P range between 10 and 30 per cent of normal was favoured. It was assumed that such a reduction of the prothrombin complex would be associated with hypocoagulability of blood of antithrombotic significance. Clinical experience indicated that a P & P reduction below 10 per cent of normal involved a definite risk of hemorrhagic complications. Borchgrevink (1960) favoured a more intensive therapy. He narrowed the therapeutic range to between 10 and 20 per cent as measured by the thrombotest method. In series of patients with angina pectoris he showed that this could be performed without an increased risk of hemorrhagic complications. Keyser (1963) maintained that an even more intensive therapy should be instituted with thrombotest as the control method. He suggested a therapeutic range from 8 to 15 per cent and showed that this could be achieved without an increased risk of bleeding. Such an intensity would roughly correspond to an increased prothrombin time of two to two-and-a-half times that of the normal by the Quick method.

When the intended anticoagulant effect of this study was settled 5 years ago it was decided to lower the upper limit from 30 to 25 per cent. It may be argued that this could safely have been lowered to 20 per cent. This might have increased the chance of obtaining maximal antithrombotic protection. But the increased risk of bleedings in these patients should be recalled. We would, therefore, have chosen the same therapeutic range today.

CHAPTER II

Survey of the Literature

A complete survey of the great number of contributions is not intended. Some recent papers, and predominantly those which in our judgement furnish fairly reliable results, are reviewed and evaluated.

Since long-term anticoagulant therapy was introduced in cerebrovascular disease as a result of its probable benefit in coronary heart disease, the current status of anticoagulants in this field is briefly reviewed.

Long term anticoagulant therapy is thought to be of value in patients with a relatively short history of angina pectoris. This quite generally accepted concept is mainly based on a single study namely that of Borchgrevink (1960 1962)

The long-term effect of anticoagulant therapy after an acute myocardial infarction is still uncertain. Apparently the question was decided during the last part of the 1950s. At that time reports appeared of the well-designed studies by Manchester (1957) Bjerkelund (1957) and The British Medical Research Council (1959 a & b) To all of these studies it is possible to raise objections. Together however they seemed to present fairly conclusive evidence of a beneficial effect from anticoagulants at least in some groups of patients. Later two Danish studies performed by Harvald *et al* (1961) and Clausen *et al* (1961) showed a favourable effect of anticoagulants on the recurrence rate of myocardial infarction, whereas the mortality rate was uninfluenced. These reports seemed to justify a less enthusiastic attitude. Recently two papers have appeared in which no effect either on the recurrence rate of coronary infarction or on the mortality rate was noted (Seaman *et al* 1964 Conrad *et al.* 1964) Apparently these studies are equally well designed and performed as those yielding opposite results. Obviously a possible benefit from anticoagulants can be neither uniform nor prominent. It may be concluded that this therapy is probably of some benefit in selected groups of patients with coronary heart disease namely in men with a relatively short coronary history who have had only one myocardial infarction. The effect is quite uncertain in women, in patients with more than one infarction, in those with a long-lasting coronary history and generally in old people.

Anticoagulants have been used to prevent further thrombo-embolism of cerebral and extracranial origin during the immediate period after the cerebral accident. In addition they have been used in ingravescent infarction Carter (1960 a & b 1961 a & b) has presented several years of extensive experience. The results of his clinical trial on patients with ingravescent infarction are of particular interest. This term means a cerebral infarction of gradual onset in which the neurological defect is still incomplete after 2 hours or more. The treated series was given initial

heparin therapy and peroral anticoagulants for 3 weeks. The final evaluation was made after 6 months and suggested that anticoagulants had improved the natural history of a progressing cerebral infarction. A lower fatality rate in the treated series was partly due to decreased mortality from pulmonary embolism. Objections may be raised to this study. The comparability of the groups was not completely documented and the double blind technique was not used.

Hansen & Lund (1962) studied the effect of peroral anticoagulant therapy during the first few weeks after the completed cerebral infarction. The effect on extracerebral thrombo-embolic complications was evident. In the treated series of 61 patients no such episode occurred, whereas extracerebral thrombo-embolism developed in 10 out of 74 cases in the control group and contributed to death in 7 of these. The double blind technique was not followed and the comparability of the two series may be questioned.

To conclude anticoagulants seem to be of some value in the treatment of patients with ingravescent infarction by hampering the progression of cerebral damage. In the completed stroke they may influence the prognosis *during the subsequent weeks of* bedrest and rehabilitation by reducing the danger of extracerebral thrombotic processes.

Studies yielding evidence in favour of a benefit from anticoagulants in cerebral infarction

A frequently overlooked defence of the use of anticoagulants in the prophylaxis of cerebral thrombosis appeared in the report by Harvald *et al.* (1962) on the long-term effect of anticoagulants after an acute myocardial infarction. The performance of this study was adequate except that a questionable randomization method was used. One hundred and forty-five patients received anticoagulants, while 170 patients were given placebo therapy. During the first year of treatment there were fewer re-infarctions as well as fewer deaths in the treated series. The difference was not of statistical significance except for the re-infarction rate in patients above 60 years of age. At the end of the second year these differences were practically eliminated. A total of 28 thrombo-embolic complications occurred in the placebo group, differing significantly from 10 cases in the treated series. This difference was mainly caused by a significantly raised number in the control series of patients with cerebral thrombosis, namely 11 against 1. No details on this point are presented. This *result is a serious argument in favour of an* anti-thrombotic effect in the vascular tree of the brain by peroral anticoagulants.

A large study of anticoagulants in cerebrovascular disease was begun at Bellevue Hospital in New York in 1956. An adequate randomization technique and placebo therapy were used. Blood control was done with the one-stage Quick method. During the following years several interim reports appeared. The first gave evidence of the comparability of the groups (McDevitt *et al.* 1959). In another report (Groch *et al.* 1959) a pronounced difference between the groups with respect to recurrent thrombo-embolic phenomena was demonstrated. In two further papers (Groch 1961, Groch *et al.* 1961) detailed documentation of the comparability of the groups was presented. Fourteen deaths in the control series were due to recurrent thrombo-embolism, while there was no such death in the treated group. At this time some doubt was introduced with respect to the comparability of the groups. For some *vaguely defined reasons*, probably of socio-economic character, the anticoagulant therapy had to be discontinued in several patients. Thus the observation period of the treated series was 890 months against

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TABLE 1 *From McDowell et al. 1963*

	Controls (100)	Treated (90) On AC	Treated (90) Off AC
Second stroke	17 (8 deaths)	1	20 (10 deaths)
Myocardial infarction	6 (4)	1	2 (1)
Pulmonary embolism	7 (7)	0	0
Peripheral vascular occlusion	1	1	1
Transient ischemic attacks	10	2	3

1 600 months of the control series. The unreserved statement was made that anticoagulants are effective in preventing further cerebral and extracerebral thrombo-embolic manifestations. In the treated series 22 per cent of the patients experienced a hemorrhagic complication. Three cases were fatal. In the apparently final report (McDowell *et al.* 1963) the treated series comprised 90 patients and the control series 100 patients. The main results are reproduced in Table 1. The death rate was slightly higher in the control group. The incidence of thrombo-embolism was much lower during periods with anticoagulant therapy. Thus the study gave strong support to the view that anticoagulants provide a prophylactic effect on recurrent thrombo-embolic phenomena in cerebrovascular disease.

Several objections to this study exist. It may be seriously questioned whether it is permissible to include observation periods *of the same patients* on and off therapy. It is not explained why the therapy was discontinued in so many patients. For those patients of the treated series in whom a recurrence developed while off therapy no information on the time of this event was presented. Thus, one cannot exclude the possibility that these thrombo-embolic phenomena were related to a 'rebound' phenomenon after cessation of the anticoagulant therapy. The double blind technique was not used and details of the anticoagulant regime were not presented. In this study comprising so many patients followed during so many years, there was only one death due to another thrombo-embolic accident. This was in marked contrast to the control series and to the treated series of other American studies. The authors of the Bellevue study offered as one explanation of the difference between their and the co-operative American studies, that the latter were performed at several hospitals involving many doctors and technicians, while the former was carried out in one hospital with a stable staff well trained in anticoagulant therapy.

Howell *et al.* (1964) reported 7 years experience of anticoagulant therapy in cere-

TABLE 2. *From Howell et al. 1964*

	Treatment series	Control series
Patients	103	92
Average follow-up period (months)	16	36
Patients suffering a recurrent stroke	7	28
Patients suffering a recurrent fatal or crippling stroke	4	26
Patients suffering recurrent stroke within 16 months	5	13

brovascular disease. Their results are reproduced in Table 2. It was concluded that anticoagulants are effective in lowering the incidence and severity of recurrent infarction in thrombo-embolic disease of the brain. Their results are impressive but difficult to evaluate because of the shortness of the report. The treated and the control series were comparable with respect to age, sex, and the frequency of hypertension. But it was not explained why the average follow-up period of the control group was more than twice that of the treated group. Their allocation method is unclear but it was stated that the distribution of patients was not made by a casual randomization technique. Placebo therapy was not used and details of the anticoagulant therapy are not presented.

Studies yielding evidence against any benefit from anticoagulants in cerebral infarction

A co-operative American study was conducted at 9 Veterans Administration Hospitals (Baker 1961). The above-mentioned objections to such an approach are also relevant to this study. However the performance and the presentation of it are among the best in this field. The patients were adequately randomized and the Quick one-stage procedure was used for blood control. Men only were studied. The patients were divided into 4 groups: treated and control patients with a T.I.A. and a completed cerebral infarction. The average observation time was 12.8 months for the control group and 9.3 months for the treated series. The groups were found comparable with respect to age and site of the cerebral lesion. But there was higher incidence of cardiac involvements in the control series. Comparison of further clinical features was not presented. Originally 155 patients were accepted for study. There were, however, several losses, including 20 deaths. At the time of the report 99 patients were distributed within the 4 groups. Anticoagulants were effective in preventing further T.I.A.s, but did not prevent the development of a cerebral infarction in these patients. In patients with a completed cerebral infarction the treatment was without effect on the recurrence rate. The mortality rate was somewhat higher in the treated group mainly because of an increased incidence of hemorrhagic complications. Four such episodes occurred. Some objections may be raised to this study. Placebo therapy was not used. The age composition is not presented and the comparability of the groups is not completely documented.

An English study on this subject was presented by Bradford Hill, Marshall & Shaw (1960, 1962). Their first report was particularly interesting, partly because this was the first adequately performed study which failed to show an effect of anticoagulants in cerebrovascular disease, and partly because the danger of this therapy was so clearly demonstrated. Patients were differentiated by sex and allocated by pairs to high and low dosage group, which received phenylindandione tablets of 50 mg and 1 mg respectively. The prothrombin level was controlled by the Quick one-stage procedure. At the time of the initial report both groups consisted of 71 patients and were equal in all important respects. There were 4 fatal cerebral hemorrhages and one fatal cardiac hemorrhagic complication in the high-dosage group against none among the controls. Although the chance of obtaining this result did not exceed the customary statistical confidence limits it was found unethical to continue the study in accordance with the original plan. It was not stated whether the study was conducted as a double blind trial and the anticoagulant therapy was not documented. Patients were included in the study without the performance of a lumbar puncture or cerebral angiography. The danger existed, therefore, of including

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TABLE 3. *From Thygesen et al.* 1964

		Anticoagulant therapy throughout the entire study	Anticoagulant therapy initially. Later placebo tablets	Placebo group during the entire study
Number of cases	109	33	35	41
Mean age	62	60	61	63
Observation period (patient years)	176	60.5	50.5	65.0
Mean observation period (months)	19	2[illegible]	17	19
No. of dead patients	11	4	3	4
No. of definite cerebrovascular accidents	25	11	7	7
No. of cerebral transitory attacks	3[illegible]	1[illegible]	8	12

omitted from their paper. Cerebral haemorrhage occurred in 3 hypertensive individual in the treated series, of whom 2 died. Eleven cerebrovascular episodes occurred in 9 patients of the treated series. On 7 of these occasions the reduction of the prothrombin complex was insufficient.

Anticoagulants in transient ischaemic attacks

Probably unjustly this condition has been considered particular category of disorders of the cerebral circulation. Quite uniformly it has been maintained that anticoagulants have a favourable effect on patients with T.I.A.s. The most comprehensive study is that of Siekert *et al.* (1961) who followed 230 patients for periods varying from one to more than 5 years. A significant effect on the frequency of T.I.A.s was noted. Of 115 patients treated continuously with anticoagulants 4 per cent developed cerebral infarction, while this happened in 40 per cent of 40 control patients. Seventy-five patients were treated for a limited period. Of these, 32 per cent had a cerebral infarction after discontinuance of therapy. This study fulfils only few of the requirements of a controlled clinical trial. The randomization procedure between the three treatment schemes is not [illegible] umentation of the com- [illegible] roups is omitted.

In the above-mentioned co-operative American studies anticoagulants were found to prevent T.I.A.s. There was, however no evidence that this therapy prevented the development of a cerebral infarction in patients with T.I.A.s. In the study of Baker *et al.* (1962) this question was difficult to decide, because the long-term prognosis of patients with transient attacks turned out to be unexpectedly favourable for both groups. Recently further evidence of the relatively benign outlook for patients with this syndrome, whether untreated or treated with anticoagulants, has been presented (Bradshaw & McQuaid 1963, Fazekas *et al.* 1963). Unlike most authors, Thygesen *et al.* (1964) were unable to demonstrate effect from anticoagulants on the recurrence rate of T.I.A.s.

Recently Gunning *et al.* (1964) produced strong evidence to support the assumption that small emboli of platelet plugs probably originating from atheromatous lesions in the arteries of the neck, may be responsible for the T.I.A.s. Since the hypocoagulability of blood induced by peroral anticoagulants is without influence on the white thrombus consisting of platelets, this theory does not explain a preventive effect from anticoagulants on T.I.A.s.

Summary

The conflicting results from various studies on the use of anticoagulants in cerebro-

cases of intracerebral hemorrhage. Autopsy revealed that this had been the case in 2 of the 4 fatal cases of cerebral hemorrhage. Since it was found probable that hypertension increased the hazards of anticoagulant therapy the trial was continued after withdrawing hypertensive individuals. In the final report the observation period had been extended to 4 years, with an average period of 28 months for the treated group and 31 months for the controls. The preliminary results on the inefficacy and danger of anticoagulants in cerebrovascular disease were confirmed

In 1958 7 medical centres in U.S A. began a co-operative study In an interim report (Fisher 1961) methodological aspects and preliminary results on the comparability of the groups were presented. The final report appeared in 1962 (Baker *et al* 1962) A total of 443 patients were observed over a period of 42 months. They were allocated at random and divided into 5 categories. transient ischemic attacks, thrombosis in evolution, thrombosis-completed stroke, thorem (thrombosis or embolism) and cerebral embolism. In most respects the treated and control patients were comparable. T I.A.s continued more frequently in the control group but anticoagulants did not *prevent* cerebral thrombosis in such patients. The treatment was without influence on the mortality rate of patients *with ingravescent* infarction but the incidence of further cerebral infarctions was probably *reduced*. Of patients with a completed stroke the treated group fared worse than the control group There were 7 severe hemorrhagic complications in the treated series, 5 of which were fatal. Another cerebral episode of probable thrombotic nature occurred *in* 5 patients (6 episodes) of *60 control* patients and in 12 patients (13 episodes) of 72 treated patients. In the thorem group *no* benefit from anticoagulants was evident and the emboli group *was too small* to permit conclusions.

The above-mentioned objections to co-operative studies are also applicable to this one. It is probably illustrative that 60 per cent of the non-fatal cases of the treated series discontinued therapy for some reason or other the majority within the first year Placebo tablets were used, but it is not stated whether the double blind technique was followed. Results of the anticoagulant therapy were not given and autopsy findings were omitted.

Howard *et al* (1963) studied the natural history of 100 patients with cerebral thrombosis during a follow-up of 3 years. The significance of hypertension and age for a poor prognosis was clearly noted. The effect of one year's anticoagulant therapy was studied in 30 patients with the double blind technique. No effect from anticoagulants was found. *The value of this study is* uncertain, since it was found extremely difficult to perform the anticoagulant therapy with sufficient intensity

An extensive Danish study was presented by Thygesen *et al.* (1964) The double blind technique was followed, but the principles of randomization were omitted in their paper *All* patients were *submitted to a lumbar* puncture and cerebral angiography The P & P method was used for blood control with an intended range from 10 to 20 per cent. The material of 109 patients were divided into 3 categories: 41 patients who received placebo tablets during the entire observation period, 35 patients who received an initial course of peroral anticoagulants which were substituted with placebo tablets after 6 weeks, and 33 patients who were treated with peroral anticoagulants throughout the study The groups were comparable with respect to age, premorbid fitness for work, and previous cerebrovascular accidents. The sex distribution and the incidence of hypertension were not presented. The main results of this study are given in Table 3. The results of the anticoagulant therapy are

TABLE 3. *From Thygesen et al. 1964*

		Anticoagulant therapy throughout the entire study	Anticoagulant therapy initially. Later placebo tablets	Placebo group during the *entire study*
Number of cases	109	33	35	41
Mean age	6–	60	61	63
Observation period (patient-years)	176	60.5	50.5	65.0
Mean observation period (months)	19	22	17	19
No. of dead patients	11	4	3	4
No. of definitive cerebrovascular accidents	25	11	7	7
No. of cerebral transitory attacks	32	12	8	12

omitted from their paper. Cerebral hemorrhage occurred in 3 hypertensive individuals in the treated series, of whom 2 died. Eleven cerebrovascular episodes occurred in 9 patients of the treated series. On 7 of these occasions the reduction of the prothrombin complex was insufficient.

Anticoagulants in transient ischemic attacks

Probably *majority* this condition has been considered particular category of disorders of the cerebral circulation. Quite uniformly it has been maintained that anticoagulants have favourable effect on patients with T.I.A.s. The most comprehensive study is that of Siekert *et al.* (1961), who followed 230 patients for periods varying from one to more than 5 years. A significant effect on the frequency of T.I.A.s was noted. Of 115 patients treated continuously with anticoagulants 4 per cent developed cerebral infarction, while this happened in 40 per cent of 40 control patients. Seventy-five patients were treated for limited period. Of these, 32 per cent had *cerebral infarction* after discontinuance of therapy. This study fulfils only few of the requirements of controlled clinical trial. The randomization *procedure* between the three treatment schemes is not presented and documentation of the comparability of the groups is omitted.

In the above-mentioned co-operative American studies anticoagulants were found to prevent T.I.A.s. There was, however no evidence that this therapy prevented the development of a cerebral infarction in patients with T.I.A.s. In the study of Baker *et al.* (1962) this question was difficult to decide, because the long-term prognosis of patients with transient attacks turned out to be unexpectedly favourable for both groups. Recently further evidence of the relatively benign outlook for patients with this syndrome whether untreated or treated with anticoagulants, has been presented (Bradshaw & McQuaid 1963 Farekas *et al.* 1963). Unlike *most authors*, Thygesen *et al.* (1964) were unable to demonstrate effect from anticoagulants on the recurrence rate of T.I.A.s.

Recently Gunning *et al.* (1964) produced strong evidence to support the assumption that small emboli of platelet plugs probably *originating* from atheromatous lesions in the arteries of the neck, may be responsible for *the* T.I.A.s. *Since the hypocoagulability* of blood induced by peroral anticoagulants is without influence on the white thrombus consisting of platelets, this theory does not explain preventive effect from anticoagulants on T.I.A.s.

Summary

The conflicting *results from various studies* on the use of anticoagulants in cerebro-

vascular disease are reviewed. Much uncertainty arises from the varied quality of the different studies which illustrates the difficulties involved in the evaluation of anticoagulants in these conditions. There is evidence that anticoagulants may have a favourable influence on the cerebral circulation in patients with an ingravescent cerebral infarction. After the completed cerebral infarction anticoagulants may improve the immediate prognosis by reducing the incidence of extracerebral thrombo-embolism. In patients with T.I.A.s anticoagulants may prevent further attacks, whereas the development of cerebral thrombosis is uninfluenced. From the available data it is difficult to assess the value of anticoagulants in the long-term treatment of patients with cerebral infarction. A careful evaluation of the conflicting results seems to indicate that anticoagulants are without effect in this condition.

Chapter III

Purpose of Study, Methods and Material

The purpose of the study was to evaluate the effect of long-term peroral anticoagulant therapy on the mortality rate, the recurrence rate, and the clinical course of patients with a cerebral infarction probably caused by thrombotic and/or atheromatous lesions of the cerebral arteries. In addition, the complications related to this treatment were recorded.

Methods

The varied symptomatology of cerebrovascular diseases their unpredictable course, and the diagnostic difficulties, make it imperative to include an adequate control group in any study of a therapeutic regime in this field. To exclude uncontrollable factors related to the belief and the prejudice of the patient and the observer placebo therapy and the double blind technique were used. Patients were allocated into the treated or the placebo group according to a preconstructed random list. Both series were submitted to the same medical and social care, including a training program, blood controls, and neurological examinations, supported by other investigations when required. The neurologist (S. B.), who was solely responsible for the neurological classification initially and during the subsequent course, was without knowledge of the distribution of patients within the groups until the end of the total observation period.

The patients, or in a few instances their relatives, were always informed by the internist (E. E.) about the experimental nature of the therapy namely that they were to be included into a clinical study which was performed to test the possible benefit of anticoagulants in cerebral stroke. In every instance the agreement to participate in such a study was obtained. It was felt impossible and from an ethical point of view unnecessary to provide them with the complete interpretation of the approach. Lay

people are without qualifications to accept the requirements of placebo therapy and the double blind technique. Evidently the patients of the control series were unaware of the character of the therapy At least, suspicion on this point was never expressed. This was accomplished by treating the patients of the two series as equally as possible. They were never informed about the result of the T T determinations. Blood controls were performed with the same frequency in both series, and in the control series adjustment of dosage was also frequently made in accordance with suppositious variations of the T T per cent. The tablets were provided free of charge to both series on bottles bearing only personal names. The placebo tablets were apparently identical to those of phenylindandione and consisted mainly of lactate and gelatine.

From a juridical and an ethical point of view this approach raises several problems. During recen years considerable literature concerning these problems has appeared, mainly however outside Scandinavia. A discussion of these aspects of controlled clinical trials exceeds the limits of this presentation. We conclude that it seems more ethical to perform a properly designed experimental study of therapeutic regime, than to make use of it in everyday practice without knowing whether it is of value or not.

The study was carried out at the Medical Department VII of Ullevål Hospital. The patients wer admitted because of a cerebrovascular accident. Some patients were admitted to the two other general medical departments (VIII & IX) of this hospital. The patients were partly admitted for diagnostic reasons, partly because of the need for immediate medical care. A history was taken and medical examination was made by one of us (E.E.). This included several blood pressure readings on subsequent days, a 12-leads electrocardiogram, an X-ray picture of the heart and the lungs, and the following blood tests: hemoglobin, white blood count, sedimentation rate, and serum concentrations of cholesterol and creatinine.

The following groups of patients were excluded from further study:

a) Patients more than 75 years old. This was partly done to prevent too heterogeneous an age composition of the material, partly because of the difficulties usually involved when performing anticoagulant therapy on an out-patient group of old people.

b) Patients who for personal (physical or mental) or geographic reasons were assumed to be unable or unwilling to co-operate.

c) Patients with repeated diastolic blood pressure readings of 120 mm Hg or more or with retinal changes of grade IV This was done because there is an increased risk of including cerebral hemorrhage in hypertensive individuals on anticoagulant therapy (Bjerkelund 1957) Hypertension is a fairly common clinical finding in patients with cerebral infarction. The complete omission of hypertensive patients would lead to a considerable reduction in the number of patients and also restrict the general validity of the study

d) Patients with hemorrhagic or xanthochromic cerebrospinal fluid. The fluid was always compared with distilled water Spinal puncture was often repeated on the following days. Hemorrhagic or xanthochromic cerebrospinal fluid may occur in cerebral infarction Such cases were also observed in this study and were excluded.

e) Patients in whom the cerebral accident could be related to extracerebral conditions with hypotension or shock, for instance acute myocardial infarction, severe bleeding episodes, infection, intoxication, etc.

vascular disease are reviewed. Much uncertainty arises from the varied quality of the different studies, which illustrates the difficulties involved in the evaluation of anticoagulants in these conditions There is evidence that anticoagulants may have a favourable influence on the cerebral circulation in patients with an ingravescent cerebral infarction. After the completed cerebral infarction anticoagulants may improve the immediate prognosis by reducing the incidence of extracerebral thrombo-embolism. In patients with T.I.A.s anticoagulants may prevent further attacks, whereas the development of cerebral thrombosis is uninfluenced. From the available data it is difficult to asses the value of anticoagulants in the long-term treatment of patients with cerebral infarction. A careful evaluation of the conflicting results seems to indicate that anticoagulants are without effect in this condition.

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acute myocardial infarction were excluded and treated in accordance with this regime In such patients the observation period was completed at the time of their myocardial infarction. Patients who had another cerebral infarction were treated in accordance with the original treatment scheme. Patients were excluded from further study if a cerebral hemorrhage occurred.

During recent years there has been considerable discussion about the so-called rebound phenomenon i.e. the possible danger of inducing a state of hypercoagulability by the discontinuance of anticoagulant therapy It is generally accepted that such therapy should be tapered off gradually As far as we know this question has never been adequately tested. With the restricted number of patients we did not expect to be able to give a definite contribution to this problem. Nevertheless, the anticoagulant therapy was terminated abruptly in every other patient while it was tapered off gradually during a 3-week period in the other half.

At the start we decided to collect at least 100 patients who would be given two years of anticoagulant or placebo therapy and thereafter be followed for at least 6 months. This intention was accomplished. The collecting period lasted 2½ years and the study was not completed until 5 years after it was started.

Material

Originally a total of 111 patients were included. Before the study we decided to exclude patients who for reasons unrelated to the cerebrovascular disorder were followed for only 3 months or less. This happened to 11 patients summarized in Table 4 The mean period of treatment was 5.5 weeks. Some of these case histories need comments.

Patient No. 26 was treated with anticoagulants for 12 weeks when symptoms and signs of the malignant disorder appeared. He died few weeks later At autopsy an old cerebral infarction was demonstrated.

Patient No. 42 was treated for one week. He should have been excluded previously because of severe hypertension.

Patient No. 48 was treated for 6 weeks when phenylindandione had to be discontinued because of a severe dermatitis caused by drug sensitivity

TABLE 4 *Patients withdrawn from the study within 12 weeks of treatment*

Patient No	Sex and age (years)	Group	Period of treatment (weeks)	Cause of exclusion
4	Female, 70	Anticoagulant	1	Died of pneumonia
14	Male 70		1	Carcinoma of the prostatic gland with metastases discovered
26	Male 71		12	Development of icterus owing to pancreatic carcinoma
42	Male 54		1	Malignant hypertension
48	Male 67		6	Dermatitis owing to drug sensitivity
50	Male, 77		12	Severe gastrointestinal bleeding of unknown site
56	Male, 53		12	Mentally unfit
65	Male, 65			—
111	Male, 59		12	—
12	Female 65	Control	2	Carcinoma of uterus discovered
35	Male, 58		1	Cerebral tumour discovered

f) Patients with a cerebral infarction presumably of embolic origin. When this study started we shared the common view that anticoagulants are of value in preventing such episodes. It was found unethical to include these patients in a clinical trial. The diagnosis can be made only with some uncertainty. We chose to exclude patients with a cerebrovascular accident of abrupt onset and associated with heart disease with or without arrhythmia. Thus patients with cerebral emboli originating from the great vessels in the neck may have been included.
g) Patients with a recent history of peptic ulcer.
h) Patients severely ill from other causes such as malignancy, severe hepatic, renal, or cardiac disease, etc.
i) Patients in whom angiography suggested a vascular anomaly or cerebral tumour.

The data on the excluded patients are not presented. By these exclusions we hoped to remain with a relatively homogeneous series of patients who were physically and mentally fit for long-term anticoagulant therapy on an out-patient basis. These were referred to the neurologist for further study. A complete neurological examination was made supported by an electroencephalogram and a cerebral angiography. The angiography was most often performed by direct catheterization of the common carotid artery, but if required, also via the femoral artery. In some instances ventriculography and isotope encephalometry were performed.

A few patients with T.I.A.s were included if the cerebral angiogram showed evidence of arteriosclerotic lesions. The distinction between a cerebral infarction and a T.I.A. may be difficult. In accordance with common principles patients with an acute episode and neurological deficits lasting for more than 24 hours were considered to suffer from cerebral infarction, while those exhibiting focal neurological signs subsiding completely within that time were considered to have had T.I.A.

We have to accept that the material might include some cases of small cerebral tumours or hemorrhages and also infarctions of embolic origin. However, it is probable that this selection furnished a series of patients the majority of whom had a cerebrovascular disorder in accordance with the purpose of the study.

When the neurologist had decided the diagnosis, the patient was given a chronological number and was allocated by the internist to the treated or the control series in accordance with a preconstructed random list.

During the subsequent course the patient was seen by the neurologist one to three times each year, or more frequently if required. In the case of recent neurological symptoms and signs a thorough examination was made as soon as possible. Most patients were submitted to another electroencephalogram and in some cases a second angiography was made.

The internist attended the patients of both series for blood control at intervals of 3 to 5 weeks, or more frequently when required. This was done predominantly on an out-patient basis, but if necessary the patients were visited in their homes. During the observation period many of the patients were hospitalized for various reasons. They were permitted at any time to apply directly to the internist or to the medical department irrespective of the ailment. During these years the internist acted as the family doctor of these patients. Although the treatment scheme was known to him, it is probable that the patients of both series were submitted to the same medical care and supervision.

At this medical department peroral anticoagulants are used during the acute phase of acute myocardial infarction and for one or two years. Patients who developed an

acute myocardial infarction were excluded and treated in accordance with this regime in such patients the observation period was completed at the time of their myocardial infarction. Patients who had another cerebral infarction were treated in accordance with the original treatment scheme. Patients were excluded from further study if a cerebral hemorrhage occurred.

During recent years there has been considerable discussion about the so-called re bound phenomenon i.e. the possible danger of inducing a state of hypercoagulability by the discontinuance of anticoagulant therapy It is generally accepted that such therapy should be tapered off gradually As far as we know this question has never been adequately tested. With the restricted number of patients we did not expect to be able to give definite contribution to this problem. Nevertheless, the anticoagulant therapy was terminated abruptly in every other patient while it was tapered off gradually during a 3-week period in the other half.

At the start we decided to collect at least 100 patients who would be given two years of anticoagulant or placebo therapy and thereafter be followed for at least 6 months. This intention was accomplished. The collecting period lasted $2\frac{1}{2}$ years and the study was not completed until 5 years after it was started.

Material

Originally a total of 111 patients were included. Before the study we decided to exclude patients who for reasons unrelated to the cerebrovascular disorder were followed for only 3 months or less. This happened to 11 *patients summarized in* Table 4 The mean period of treatment was 5.5 weeks. Some of these case histories need comments.

Patient No. 26 was treated with anticoagulants for 12 weeks when symptoms and signs of the malignant disorder appeared. He died few weeks later At autopsy an old cerebral infarction was demonstrated.

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48	Male 67		6	Dermatitis owing to drug sensitivity
50	Male, 57		12	Severe gastrointestinal bleeding of unknown site
56	Male, 53		12	Mentally unfit
65	Male, 65		1	—•—
111	Male, 59		12	—•—
12	Female, 65	Control	2	Carcinoma of uterus discovered
35	Male, 58		1	Cerebral tumour discovered

He did well until 3 months later when he was killed in a road accident. Post-mortem examination was not performed.

Patient No 50 experienced a severe gastro-intestinal bleeding requiring blood transfusions. Two weeks previously he had a T T per cent of 18 and on the day of the bleeding it was 22. Roentgenological examination of the gastrointestinal tract was negative. He has now been well for 3 years without new symptoms.

Patients Nos. 56, 65 and 111 were thought to be adequately co-operable, but the next few weeks of therapy were unsuccessful. The fate of patient No. 65 is unknown, while the other two are doing well *without new cerebrovascular episodes*.

It may be questioned whether the 5 patients treated for periods from 6 to 12 weeks should be included. In our opinion an anti-

TABLE 5 *Sex distribution*

	Treated series		Control series	
	Females	Males	Females	Males
No. of patients	23	28	15	34
Per cent	45 1	54.9	30.6	69 4

TABLE 6. *Age distribution*

	Treated series			Control series		
	Females	Males	Both sexes	Females	Males	Both sexes
Mean age (years)	63 7	60.2	61 7	64.0	63.3	63.5
S D	6.1	7.3	7.0	8.5	7.3	7.6
Range	49-72	46-70	46-72	48-74	47 74	47-74

TABLE 7 *Serum cholesterol level and overweight*

	Treated series			Control series		
	Females	Males	Both sexes	Females	Males	Both sexes
Mean serum cholesterol (mg/100 ml)	306	261	281	280	278	279
S.D (mg/100 ml)	76	64	70	40	50	47
No. of patients with serum cholesterol above 350 mg/100ml	4	3	7	0	3	3
Overweight, No of patients	5	1	6	6	6	12

TABLE 8 *Frequency and severity of hypertension*

	Treated series		Control series	
	Females	Males	Females	Males
Diastolic blood pressure bove 95 mm Hg No. of patients	18	[illegible]	8	14
Hypertensive heart disease or retinal changes of grade III, No. of patients	6	4	2	8
No. of patients on *hypotensive therapy*	5		2	4

coagulant regime should exceed this period at least until conclusions of its 'long-term efficiency are drawn. These patients are therefore omitted from the subsequent presentation. Patients Nos. 48 and 50 however are included in the survey of complications to the anticoagulant therapy

The final material consisted of 100 patients of whom 51 belonged to the treated and 49 to the control series. Tables 5 to 8 present data of general medical interest. In both series, and particularly among the controls, males predominated. But the different sex ratio of the groups is not of statistical significance. In both series the mean ages of females and males were in the range of 60 to 64 years and did not differ significantly from each other. Serum cholesterol was measured by the method of Carr & Drekter (1956) The mean levels are shown in Table 7 No significant differences between the groups or the two sexes are noted. Both sexes show somewhat higher mean values than the corresponding values of presumably healthy Norwegian individuals of the same age (Kirkeby 1965). The moderate preponderance in the treated series of patients with particularly high serum cholesterol levels is not of statistical significance. The same applies to the uneven distribution between the groups of patients with overweight, which in this study means a weight exceeding 15 per cent of normal according to Norwegian height/weight Tables (Lindberg *et al.* 1956). In this study hypertensive individuals are those with repeated diastolic blood pressure readings on

TABLE 9 *Neurological features at the beginning of the treatment*

			Number of patients			
			Treated series		Control series	
Diagnosis	Cerebral infarction	One insult	41	50	34	47
		Several insults	9		13	
	Transitory ischemic attacks	One episode	1	1	2	2
		Several episodes	0		0	
Clinical localization	Area of the anterior and/or the middle cerebral artery		41		37	
	Area of the vertebral/basilar or the posterior cerebral artery		10		12	
Angiography	Not performed		2		2	
	Normal findings		13		18	
	Delayed circulation		4		3	
	Atheromatous lesions	Extracranial	5	9	9	9
		Intracranial	4		0	
	Stenosis	Extracranial	9	14	8	9
		Intracranial	5		1	
	Occlusion	Extracranial	5	9	6	8
		Intracranial	4		2	
Electroencephalography	Normal activity		17		19	
	Pathological activity	Diffuse	6	34	7	30
		Local	28		23	

He did well until 3 months later when he was killed in a road accident. Post-mortem examination was not performed.

Patient No. 57 experienced a severe gastrointestinal bleeding requiring blood transfusion. Two weeks previously he had a TT per cent of [illegible] and on the day of the bleeding it was 22. Roentgenological examination of the gastrointestinal tract was negative. He has now been well for [illegible] years without new symptoms.

Patients Nos. 36, 65, and 111 were thought to be adequately co-operable, but the next few weeks of therapy were inadequate. The fate of patient No. 65 is unknown, while the other two are doing well without new cerebrovascular episodes.

It may be questioned whether the 5 patients treated for periods from 6 to 12 weeks should be included. In our opinion an ans-

Table 5. *Sex distribution*

	Treated series		Control series	
	Females	Males	Females	Males
No. of patients	23	28	15	34
Per cent	45.1	54.9	30.6	69.4

Table 6. *Age distribution*

	Treated series			Control series		
	Females	Males	Both sexes	Females	Males	Both sexes
Mean age, years	63.7	62.2	62.7	64.0	63.3	63.5
S.D.	6.3	7.3	7.0	6.5	7.3	7.6
Range	[illegible]	45–70	[illegible]	49–74	47–74	47–74

Table 7. *Serum cholesterol, lipids and overweight*

	Treated series			Control series		
	Females	Males	Both sexes	Females	Males	Both sexes
Mean serum cholesterol, mg/100 ml	[illegible]	[illegible]	283	[illegible]	275	279
S.D. mg/100 ml	76	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
No. of patients with serum cholesterol above 350 mg/100 ml	4	[illegible]	–	0	[illegible]	[illegible]
Overweight, no. of patients	5	1	6	6	[illegible]	[illegible]

Table 8. *Frequency and severity of hypertension*

	Treated series		Control series	
	Females	Males	Females	Males
Diastolic blood pressure above 95 mm Hg, No. of patients	18	—	6	14
Hypertensive heart disease or retinal changes of grade III, no. of patients	6	4	–	6
No. of patients on hypotensive therapy	5	2	–	4

The muscle strength was graduated from 0 to 5 (full strength) and the individual patient classified according to the functional status of the maximal affected muscle group

The term functional incapacity is based on complete evaluation of the patients ability to attain the standard of health and strength required by everyday life outside hospital. Of course, such a separation into 5 functional categories is a questionable differentiation. However it should be kept in mind that this was done by the neurologist, who was unaware of the distribution of patients within the groups.

The Tables reveal slight preponderance in the treated series of severely disabled patients. Thus 13 patients of the treated series had a muscle strength of grade 0 while 32 of them were 100 per cent incapacitated. In the control series the corresponding figures were 8 and 27 patients respectively In the treated series 13 patients had a normal angiogram, while 17 patients showed a normal electroencephalogram. The corresponding figures of the control series were 18 and 19 patients respectively These slight divergences are far from being of statistical significance.

It seems justified to conclude that the patients of the two series were comparable in all important general medical and neurological respects, and that the case material furnished an adequate base for the evaluation of anticoagulant therapy

Chapter IV

Results

Anticoagulant therapy

Apart from data demonstrating the comparability of the groups it is important to present evidence that the patients of the treated and the control series really differed with respect to the hypocoagulability of blood effected by the anticoagulant regime

It was intended to reduce the prothrombin complex of blood within 10 and 25 per cent of normal, as measured by the thrombotest method (Owren 1959) During the stay in hospital the T T determinations were performed once or twice weekly During the subsequent course this was done at intervals of 3 to 5 weeks, or more frequently when required.

Previous authors concerned with such data (Bjerkelund 1959 Borchgrevink 1960) based their presentation upon the results of the total number of the P & P or T T determinations. Usually such determinations are made at shorter intervals during periods with the T T per cent at the two extremes of the therapeutic range. Therefore, it was found more useful to calculate the approximate T T per cent of the single week during the treatment period of each patient. Table 11 and Figures 1 and 2 are based on the total number of such weekly T T percentages.

The mean treatment period of the 51 patients was 22.8 months during total of 1,162 patient-months (Table 21) Forty three patients were treated for 20 months or more. The grand mean of the individual mean weekly T T percentages was 18.6 per cent. Only 4 patients or 7.8 per cent had mean weekly T T per cent above 25, namely 26, 27 31 and 32 respectively Three

TABLE 10 *Neurological findings at the beginning of the treatment period*

		Number of patients	
		Treated series	Control series
Mental disturbances		5	4
Aphasia		19	13
Cranial nerve signs		43	39
Dysarthria		24	23
Motor function (muscle strength grade 0 5)	Grade 0	13	8
	1 3	15	8
	4-5	23	33
Cerebellar signs		11	10
Sensory signs		20	18
Reflex changes		47	44
Functional incapacity (0 100)	100 %	32	77
	75 %	4	5
	50 %	3	9
	25	6	4
	0	6	4
Epilepsy		1	0

subsequent days exceeding 95 mm Hg All hypertensive individuals were examined by an ophthalmologist and the fundi recorded according to the Keith–Wagener classification. The diagnosis of hypertensive heart disease was based on an evaluation of clinical, roentgenological, and electrocardiographic findings. Table 8 shows the frequency and the severity of hypertension of the two series. No difference of statistical significance between the groups is observed. However 68 per cent of the females of the total case material were hypertensive against 45 per cent of the males. This difference is of statistical significance (chi square 4.24 i.e $0.05 > p > 0.02$)

In addition, 4 patients of the treated series suffered from diabetes mellitus of moderate severity while there were 2 such cases in the control series. In the latter there was also one case of chronic nephritis with hypertension and moderate renal insufficiency

Tables 9 and 10 summarize neurological features at the beginning of the treatment period. One patient of the treated series and 2 patients of the control series were included because of a T.I.A. The remainder had a completed stroke. In both series the average period from the onset of clinical symptoms until the start of therapy was about 20 days (Table 21)

Angiography of the actual arterial territory was performed as indicated by the clinical signs. The results were classified according to the most prominent arterial lesions present. The preponderance of extracranial arterial lesions is interesting A detailed presentation and discussion of this point and other angiographic aspects exceed the limits of this paper

About two-thirds of the patients presented pathological electroencephalographic findings predominantly of localized character Of 22 patients with lesions of the basilar/vertebral or posterior cerebral arteries, 13 had a normal electroencephalogram, while angiography was negative in 6 of them.

The term mental disturbances is used to characterize the patients who deviated from their previous intellectual or emotional behaviour

The muscle strength was graduated from 0 to 5 (full strength), and the individual patient classified according to the functional status of the maximal affected muscle group.

The term functional incapacity is based on complete evaluation of the patients ability to attain the standard of health and strength required by everyday life outside hospital. Of course, such a separation into 5 functional categories is a questionable differentiation. However it should be kept in mind that this was done by the neurologist, who was unaware of the distribution of patients within the groups.

The Tables reveal slight preponderance in the treated series of severely disabled patients. Thus 13 patients of the treated series had a muscle strength of grade 0 while 32 of them were 100 per cent incapacitated. In the control series the corresponding figures were 8 and 27 patients respectively In the treated series 13 patients had a normal angiogram, while 17 patients showed a normal electroencephalogram. The corresponding figures of the control series were 18 and 19 patients respectively These slight divergences are far from being of statistical significance.

It seems justified to conclude that the patients of the two series were comparable in all important general medical and neurological respects, and that the case material furnished an adequate base for the evaluation of anticoagulant therapy

CHAPTER IV

Results

Anticoagulant therapy

Apart from data demonstrating the comparability of the groups it is important to present evidence that the patients of the treated and the control series really differed with respect to the hypocoagulability of blood effected by the anticoagulant regime.

It was intended to reduce the prothrombin complex of blood within 10 and 25 per cent of normal, as measured by the thrombotest method (Owren 1959) During the stay in hospital the T T determinations were performed once or twice weekly During the subsequent course this was done at intervals of 3 to 5 weeks, or more frequently when required.

Previous authors concerned with such data (Bjerkelund 1959 Borchgrevink 1960) based their presentation upon the results of the total number of the P & P or T T determinations. Usually such determinations are made at shorter intervals during periods with the T T per cent at the two extremes of the therapeutic range. Therefore it was found more useful to calculate the approximate T T per cent of the single week during the treatment period of each patient. Table 11 and Figures 1 and 2 are based on the total number of such weekly T T percentages

The mean treatment period of the 51 patients was 22.8 *months during* a total of 1 162 patient-months (Table 21). Forty-three patients were treated for 20 months or more. The grand mean of the individual mean weekly T T percentages was 18.6 per cent. Only 4 patients or 7.8 per cent had a mean weekly T T per cent above 25 namely 26, 27 31 and 32 respectively Three

TABLE 11 *Data on the individual anticoagulant therapy*

Patient No.	Duration of therapy (weeks)	Mean weekly T T per cent	S.D. of mean weekly T T per cent	Percentage of weeks with T T per cent above 25	Percentage of weeks with T T per cent below 10
1	97	18	8	18	18
2	63	22	9	37	10
9	26	13	4	8	19
10	95	26	14	41	4
11	117	22	11	31	8
15	96	12	4	1	31
18	40	27	6	43	0
19	100	18	4	8	0
21	111	18	6	7	5
24	102	21	10	26	0
25	99	16	7	16	21
28	110	22	9	41	5
29	101	23	9	26	0
30	106	21	8	22	0
31	94	20	6	23	0
32	87	17	7	16	13
36	103	21	11	21	14
38	95	16	7	10	36
40	15	32	10	60	0
43	27	31	7	74	0
46	10[illegible]	15	7	7	17
54	95	19	9	26	26
55	101	19	8	10	4
58	99	17	8	19	16
59	99	16	6	9	13
66	16	14	3	0	0
67	77	16	8	12	12
69	99	19	8	18	7
70	100	17	5	1	4
73	99	17	6	6	10
74	98	17	8	8	18
76	100	19	5	3	4
77	39	14	6	5	33
79	98	20	9	26	11
82	109	14	5	4	24
83	99	16	4	0	5
84	94	18	8	19	11
85	120	16	4	0	0
86	100	16	4	5	0
88	83	20	12	8	16
91	108	20	8	18	4
95	106	15	3	0	0
96	109	15	4	0	4
97	116	22	8	20	0
99	105	19	7	19	5
101	107	17	4	3	8
102	109	19	4	6	0
103	105	16	4	3	1
104	109	15	3	0	4
106	103	20	3	4	0
110	100	18	10	16	22
Mean	9[illegible]0	18.6	6.8	15 7	9 1
Standard deviation	25 9	4.0	[illegible]6	15 7	9.6
Range	15-120	12-32	3-14	0-74	0-36

of these patients were treated during a relatively short period, 15 27 and 40 weeks respectively During 14 0 per cent of the total treatment period of all patients the T T per cent was above 25 while it was below 10 during 8 9 per cent of the period.

Further details on the anticoagulant regime are given in connection with the patients who experienced a cerebral or coronary infarction or a bleeding episode.

In the control series a total of 1 102 T T determinations were performed. The results are presented in Figure 3 The median of the values is 85 per cent. The configuration of the diagram is related to the fact that no differentiation was made of T T values above 100 per cent.

Treatment period

Cerebrovascular phenomena and neurological status

When a cerebrovascular episode occurred

FIGURE 1. *Distribution of the mean weekly T T per cent*

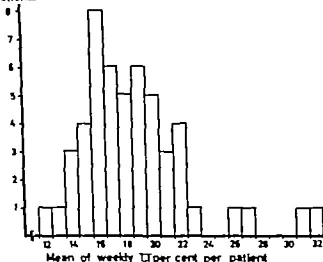


FIGURE 2. *Number of weeks within different T T ranges expressed as per cent of the total anticoagulant period*

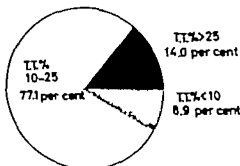
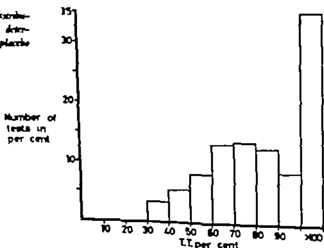


FIGURE 3. *Percentage distribution of 1,102 T T determinations in the placebo patients*



the diagnostic methods and criteria were the same as those used initially Sometimes it was found difficult to distinguish between a T I.A and an epileptic attack of other etiology When in doubt convulsions and unconsciousness favoured the diagnosis of epilepsy while transitory neurological defects were found indicative of a T I.A

A cerebral stroke was registered as fatal if death took place within the first 2 weeks of onset

Tables 12 and 13 show the patients in whom the anticoagulant or placebo therapy was discontinued earlier than intended. In the treated series there were 9 such patients against 11 of the control series. In 2 patients of both groups the therapy had to be discontinued due to mental deterioration. Two males of the control series and one of the treated series had an acute myocardial infarction and were excluded from further study according to the rules mentioned previously

Table 14 gives the number of patients who experienced a cerebrovascular accident during the treatment period. In the treated series there was one fatal case of cerebral infarction against 3 in the control series. In the treated series 5 cerebral infarctions occurred during a total of 1 162 patient-months, i.e. 4.3 per 1 000 observation months. In the control series 11 cerebral infarctions occurred in 10 patients during 1 105 patient-months, i.e. 10.0 episodes per 1 000 observation months The difference between the number of patients who suffered another cerebral infarction is not of statistical significance (chi square 1 45 $p = 0.23$) The result is not altered if corrections are made for differences in the length of observation

The sex distribution is presented in Table 5 Table 14 shows a predominance of recurrent infarctions in the males of both series. This happened in 4 of 28 males in the treated series and in 9 of 34 males among the controls. This difference is not of statistical significance. If the two series are treated separately irrespective of the treatment scheme, another cerebral infarction occurred in 2 of 38 females and in 13 of 62 males. This difference is not far from being of statistical significance (chi square 3 40 $0.10 > p > 0.05$) and suggests a poorer prognosis in males.

The two series did not differ significantly with respect to the occurrence of a T.I.A. This happened in 6 patients of the control series and in 4 patients of the treated series. In 8 of these cases the original cerebral lesion had been in the area of the anterior or the middle cerebral artery A total number of 9 cerebral infarctions and T I.A.s occurred in the treated series against 21 among the controls. These were distributed as follows.

	Treated series	Control series
Patients with 1 episode	9	11
Patients with episodes	0	5

This uneven distribution is statistically significant (chi square for trend 5 12, $0.05 > p > 0.02$)

Apart from the patients who experienced a myocardial infarction no other episode of extracerebral thrombo-embolism occurred.

Table 9 demonstrates the considerable number of patients in both series with angiographic evidence of a stenotic lesion of the extracranial portion of the cerebral arteries. It is probable that such patients are inclined to develop a complete occlusion by a thrombotic process. In these patients, therefore a particular benefit from anticoagulants might be expected. However the present study gives no support to this assumption. Three of the 5 treated patients who experienced a recurrent cerebral infarction had an extracranial stenotic lesion of the cerebral arteries compared to 6 of the control patients who had a recurrence. Thus, the grave significance of such lesions is suggested, whereas there was nothing to indicate a protection from anticoagulants.

Table 12. *Patients in whom the anticoagulant therapy was terminated earlier than intended*

Sex and age (years)	Period of anticoagulant therapy (months)	Cause of termination
Male, 63	16	Died of cerebral infarction
Male, 62	9	Died of cerebral hemorrhage
Female, 66	20	-"- -"-
Female, 68	4	-"- -"-
Female, 61	6	Dead. Did not awake from general anesthesia after operation for perforated gastric ulcer
Female, 72	7	Mental disturbances
Female, 70	6	-"-
Male, 66	10	Died of enterocolitis caused by staphylococci
Male, 49	26	Acute non-fatal myocardial infarction

Table 13. *Patients in whom the placebo therapy was terminated earlier than intended*

Sex and age (years)	Period of placebo therapy (months)	Cause of termination
Male, 51	15	Died of cerebral infarction
Male, 76	19	-"- -"-
Male, 73	7	-"- -"-
Male, 63	18	Sudden death (autopsy not performed)
Male, 70	13	Died of pneumonia 2 months after another cerebral infarction
Male, 70	12	Died of pneumonia 5 months after another cerebral infarction
Female, 76	17	Mental disturbances
Female, 70	13	-"-
Male, 69	14	Carcinoma of colon with metastases
Male, 50	18	Acute non-fatal myocardial infarction
Male, 48	11	-"- -"- -"-

Table 14. *Number of patients experiencing cerebrovascular episode during anticoagulant or placebo therapy. The figures in parentheses indicate number of episodes*

	Treated series			Control series		
	Females	Males	Both sexes	Females	Males	Both sexes
Non-fatal cerebral infarction	1	3	4	1	6 (7)	7 (8)
Fatal cerebral infarction	0	1	1	0	3	3
Fatal cerebral hemorrhage	2	1	3	0	0	0
Transitory ischemic attacks	0	4	4	3 (5)	3 (5)	6 (10)

3

the diagnostic methods and criteria were the same as those used initially Sometimes it was found difficult to distinguish between a T I A. and an epileptic attack of other etiology When in doubt convulsions and unconsciousness favoured the diagnosis of epilepsy while transitory neurological defects were found indicative of a T I A.

A cerebral stroke was registered as fatal if death took place within the first 2 weeks of onset.

Tables 12 and 13 show the patients in whom the anticoagulant or placebo therapy was discontinued earlier than intended. In the treated series there were 9 such patients against 11 of the control series In 2 patients of both groups the therapy had to be discontinued due to mental deterioration Two males of the control series and one of the treated series had an acute myocardial infarction and were excluded from further study according to the rules mentioned previously

Table 14 gives the number of patients who experienced a cerebrovascular accident during the treatment period. In the treated series there was one fatal case of cerebral infarction against 3 in the control series. In the treated series 5 cerebral infarctions occurred during a total of 1 162 patient-months, i.e. 4.3 per 1 000 observation months. In the control series 11 cerebral infarctions occurred in 10 patients during 1 105 patient months, i.e. 10 0 episodes per 1 000 observation months The difference between the number of patients who suffered another cerebral infarction is not of statistical significance (chi square 1 45 $p = 0.23$) The result is not altered if corrections are made for differences in the length of observation

The sex distribution is presented in Table 5 Table 14 shows a predominance of recurrent infarctions in the males of both series. This happened in 4 of 28 males in the treated series and in 9 of 34 males among the controls. This difference is not of statistical significance. If the two sexes are treated separately irrespective of the treatment scheme, another cerebral infarction occurred in 2 of 38 females and in 13 of 62 males. This difference is not far from being of statistical significance (chi square 3.40 $0\ 10 > p > 0\ 05$) and suggests a poorer prognosis in males.

The two series did not differ significantly with respect to the occurrence of a T.I A. This happened in 6 patients of the control series and in 4 patients of the treated series. In 8 of these cases the original cerebral lesion had been in the area of the anterior or the middle cerebral artery A total number of 9 cerebral infarctions and T I.A.s occurred in the treated series against 21 among the controls. These were distributed as follows.

	Treated series	Control series
Patients with 1 episode	9	11
Patients with 2 episodes	0	5

This uneven distribution is statistically significant (chi square for trend 5.12, $0\ 05 > p > 0\ 02$)

Apart from the patients who experienced a myocardial infarction no other episode of extracerebral thrombo-embolism occurred.

Table 9 demonstrates the considerable number of patients in both series with angiographic evidence of a stenotic lesion of the extracranial portion of the cerebral arteries. It is probable that such patients are inclined to develop a complete occlusion by a thrombotic process. In these patients, therefore, a particular benefit from anticoagulants might be expected. However the present study gives no support to this assumption. Three of the 5 treated patients who experienced a recurrent cerebral infarction had an extracranial stenotic lesion of the cerebral arteries compared to 6 of the control patients who had a recurrence. Thus, the grave significance of such lesions is suggested, whereas there was nothing to indicate a protection from anticoagulants.

another cause before the neurologist was able to examine them. Such patients were classified according to the last record prior to the accident. When the data of Table 16 are compared with those of Table 10 a moderate general improvement of the neurological status of both series is noted. The originally slight preponderance in the treated series of more severely disabled patients is probably still reflected in Table 16 since 33 patients of this series had a muscle strength of grade 4 to 5 against 40 of the control patients. On the other hand, the two series now contained a similar number of 100 per cent incapacitated patients. A comparison of the data presented in Tables 10 and 16 justifies the conclusion that there was no difference between the series with respect to the improvement of the neurological status during anticoagulant and placebo therapy

Complications to the anticoagulant therapy

These are summarized in Table 17 One patient was excluded from the study owing to a severe drug dermatitis. A total of 10 bleeding episodes were recorded. Three moderately hypertensive patients had an acute spontaneous cerebral hemorrhage followed by death a few hours later This happened 4 9 and 21 months respectively after the start of the therapy In one of them a low T T per cent of 9 was noted at the time of the episode, while in the other two the percentages were 16 and 27 One patient had a severe gastrointestinal bleeding and was excluded after 12 weeks. The remaining 6 patients had only minor bleedings. Apart from a reduction of the anticoagulant dosage in 3 of them the hemorrhages were of no consequence.

Post-treatment period

Prior to the study it was decided to follow the patients for at least 6 months after completion of the treatment. Since the collection period lasted longer than expected the post treatment observation period became relatively long and averaged 14 7 and 16.7

TABLE 17 *Complications related to the anticoagulant therapy*

Sex and age (years)	Hypertension present	T T per cent at the time of complication	Kind and severity of complication	Consequences
Male, 62	Yes	16	Cerebral hemorrhage	Died
Female 66		9	—	
Female, 68		27	—	
Male 69	No	9	Moderate spontaneous subcutaneous bleeding	None
Female, 68		7	Moderate hematuria. Site not discovered	
Male 66	Yes	19	Moderate subconjunctival bleeding	
Male, 64		14	Moderate epistaxis	
Male, 45	No	12	Moderate intramuscular bleeding	
Female, 70	Yes	11	Moderate gastrointestinal bleeding from undiscovered site	
Male 63	No	22	Severe gastrointestinal hemorrhage from undiscovered site requiring blood transfusions and vitamin K injections	Excluded from study after 12 weeks
Male, 69	No		Severe allergic dermatitis	—

TABLE 15 *The intensity of the anticoagulant therapy and the occurrence of cerebrovascular episodes*

Sex and age (years)	Treatment period until episode (months)	Cerebrovascular disturbance	Time from the last T T determination to the episode (weeks)	The last T T value (per cent)	T T value on the day of episode (per cent)
Male 63	16	Fatal cerebral infarction	2	12	9
Female 71	5	Non-fatal cerebral infarction	2	18	13
Male 66	18	—"— —"—	4	10	10
Male 60	12	—"— —"—	2	18	21
Male 66	13	—"— —"—	2	17	17
Male 68	15	T.I.A.	1	20	20
Male 72	22	—"—	2	18	15
Male 55	12	—"—	2	18	15
Male 49	26	T.I.A. probably induced by acute myocardial infarction	1	20	13

TABLE 16. *Neurological findings at termination of the treatment period*

		Number of patients	
		Treated series	Control series
Mental disturbances		9	10
Aphasia		13	9
Cranial nerve signs		32	23
Dysarthria		11	10
Motor function (muscle strength grade 0-5)	Grade 0	6	3
	1-3	12	6
	4-5	33	40
Cerebellar signs		4	9
Sensory signs		15	15
Reflex changes		40	43
Functional incapacity (0-100 %)	100 %	19	18
	75 %	3	5
	50 %	9	5
	25 %	6	8
	0	14	13
Epilepsy		3	5

Table 15 shows the intensity of the anticoagulant regime during periods of a cerebral infarction or a T I.A. In every one of the 9 patients the T T per cent was in the range between 9 and 21 during such episodes

Table 16 summarizes the neurological findings at the termination of the anticoagulant and placebo therapy The great majority of the patients were examined close to the conclusion of therapy Some patients, however died of a cerebrovascular accident or

TABLE 20. *The total number of patients experiencing cerebrovascular accident or myocardial infarction during anticoagulant or placebo therapy and during the post-treatment observation period. Asterisks indicate death during the acute phase. The line after the 6th post-treatment month indicates the minimal observation period*

Time from start of treatment (months)	Treated series					Control series			
	Cerebral infarction		Fatal cerebral hemorrhage	T.I.A.	Acute myocardial infarction	Cerebral infarction		T.I.A.	Acute myocardial infarction
	Fatal	Non-fatal				Fatal	Non-fatal		
0-3									
4-6		1	1			1	1		
7-9			1				3	1	
10-12		1		1				2	1
13-15		1		1		1		2	
16-18	1	1					1		1
19-21			1			1		1	
22-24				1			2		
25-26				1	1				
Time from start of the post-treatment period (months)	'Treated' series					'Control' series			
0-3				2	1		2	1	
4-6		1		1	2*		1	1	
7-9	1	1			1				
10-12				1					
13-15									
16-18		1					2		1

TABLE 21 *Period from the acute cerebrovascular accident until start of therapy, the duration of anticoagulant or placebo treatment, and the total observation period*

	Treated series	Control series
Mean period ± S.D. and range from acute cerebrovascular accident until start of therapy (days)	20.7 ± 26.1 (2-180)	20.1 ± 4.3 (2-90)
Mean period ± S.D. and range of anticoagulant or placebo therapy (months)	22.8 ± 5.9 (4-27)	22.6 ± 4.7 (7-31)
Total number of patient-months on anticoagulant or placebo therapy	1.162	1.105
Mean period ± S.D. and range of observation after cessation of anticoagulant or placebo therapy (months)	14.7 ± 10.9 (0-34)	16.7 ± 10.7 (0-32)
Total number of patient-months after cessation of anticoagulant or placebo therapy	752	819
Mean ± S.D. and range of total observation period (months)	37.5 ± 13.9 (4-59)	39.3 ± 13.6 (7-59)
Total number of patient-months	1,914	1,924

TABLE 18. *Number of patients experiencing a cerebrovascular episode after cessation of anticoagulant or placebo therapy. The figures in parentheses indicate number of episodes*

	Treated series			Control series		
	Females	Males	Both sexes	Females	Males	Both sexes
Non-fatal cerebral infarction	0	3 (4)	3 (4)	1	4	5
Fatal cerebral infarction	0	1	1	0	0	0
Fatal cerebral hemorrhage	0	0	0	0	0	0
Transitory ischemic attacks	2 (3)	2 (4)	4 (7)	1	1	2

months for the treated and the control series respectively. Detailed data are presented in Table 21. During this period the neurologist was still unaware of the group to which the individual patient had belonged. If a cerebrovascular episode occurred the same diagnostic methods and criteria as before were used. Table 18 shows the number of patients who experienced a cerebrovascular accident during this period. In the originally treated series there was one fatal case of a cerebral infarction while 3 other patients had a total of 4 non-fatal cerebral infarctions, i.e. 6.6 infarctions per 1 000 observation-months. In the control series there were no fatal episodes, while 5 patients experienced a similar number of non-fatal cerebral infarctions, i.e. 6.1 per 1 000 observation-months. Four patients of the treated series had a T.I.A. during this period, against 2 of the control series.

Thus, there was no difference between the series with respect to the occurrence of cerebrovascular episodes during this part of the study.

Table 19 gives the clinical or pathological localization of the total number of 26 recurrent cerebral infarctions of the two series. During the treatment period there was a preference for an ipsilateral localization of the recurrence whereas there was no such tendency during the post-treatment period.

Table 20 illustrates the distribution within the treatment and post-treatment periods of the cerebral and cardiac episodes of both series. The number of patients experiencing such events is presented. Cerebral hemorrhages occurred exclusively in the treated series during the anticoagulant period. Although there is no difference of statistical significance the Table shows a moderate accumulation of cerebral infarctions in the

TABLE 19. *Localization of recurrent cerebral infarctions compared with original lesion*

		No. of episodes			
		Treated series		Control series	
		Same side	Contra-lateral side	Same side	Contra-lateral side
Treatment period	Non-fatal cerebral infarction	4	0	7	1
	Fatal cerebral infarction	1	0	1	2
Post-treatment period	Non-fatal cerebral infarction	3	1	3	2
	Fatal cerebral infarction	0	1	0	0

TABLE 23. *Data on the patients who survived the observation period while still under observation*

		Treated series			Control series	
	Females	Males	Both sexes	Both sexes	Females	Males
No. of patients	14	18	32	32	12	20
No. of patients with hypertension	11	6	17	14	8	6
Mean age ± S.D. and range			64.1 ± 7.1 (43–75)	65.8 ± 7.4 (48–78)		
Degree of functional incapacity at the start of the study (No. of patients)		100 %	19	17		
		75 %	2	4		
		50 %	1	7		
		25 %	6	2		
		0	4	2		
Degree of functional incapacity at the end of the observation period (No. of patients)		100 %	13	9		
		75 %	0	3		
		50 %	6	4		
		25 %	3	7		
		0	10	9		

moderate preponderance of 100 per cent incapacitated individuals compared to the control' series.

Table 23 gives data on the 32 patients of both series who were alive and under observation at the completion of the study. Two additional patients were alive, namely one male of the treated series and one male of the control series, who had both been excluded because of myocardial infarction. The Table demonstrates the good conformity between the groups. A moderate improvement of the neurological status of both series had occurred during the observation period.

Fatalities

Clinical aspects

Of the total number of 100 patients, 34 died during the observation period, which aver aged 3 2 years. Of these, 18 patients belonged to the treated series and 16 to the control series. Sixteen deaths in the treated series and 11 deaths in the control series occurred during the proper observation period.

Table 24 presents 6 patients who died during the anticoagulant period. In 5 of these the fatal outcome was more or less related to the cerebrovascular disorder. This gives a mortality rate of 4.3 per 1,000 observation-months.

Table 25 gives data on 6 patients who died during the placebo period. Patients Nos. 4 and 5 died of pneumonia after they had deteriorated because of recurrent cerebral infarction some months previously. It is reasonable to consider all these deaths as more or less related to the cerebrovascular disorder. This gives mortality rate of 5.4 per 1,000 observation-months.

Thus there was an equal mortality rate of the two series during the anticoagulant and placebo period.

Table 26 presents data on the originally treated patients who died during the post-treatment period. Four of the 10 deaths were more or less related to the cerebrovascular

control series during the treatment period and of myocardial infarctions in the treated series during the post-treatment period. The latter phenomenon is of particular interest owing to the possible influence of a *rebound phenomenon*. Anticoagulant treatment was *terminated abruptly* in one-half of the patients and tapered off gradually during a three-week period in the other half. During the first three months after cessation of therapy there was no recurrence in the treated series, while two cerebral *infarctions* occurred in the control series. However a 66-year-old man suddenly died 3 weeks after the abrupt termination of the anticoagulant therapy. At autopsy a recent coronary thrombus was demonstrated. In 3 other cases of the originally treated series a fatal myocardial infarction occurred later *in the* post-treatment period. In all of them the therapy had been *tapered off gradually*. The same regime was followed in the 2 patients of the treated series who experienced a T.I.A. during the first 3 months after cessation of therapy. Thus, this study does not advocate a gradual termination of anticoagulant therapy.

Table 22 presents the neurological findings at the end of the observation period. The treated series consisted of 45 patients and the control series of 43. This was caused by the exclusion of 6 patients of each group who *died during* the anticoagulant or placebo period. These were included in Table 16. Table 22 includes 3 patients of the anticoagulant series and 5 patients of the placebo series in whom the treatment and observation period were concluded earlier than intended owing to reasons specified in Tables 12 and 13. *These were listed with the* same status in Table 22 as in Table 16. When Table 22 is compared to Tables 10 and 16 it is observed that the treated series still contained a smaller number of patients with a muscle strength of grade 4 to 5 than the *control series. However, at variance with the* situation at the conclusion of the treatment period the treated series now showed a

TABLE 22. *Neurological findings at the end of the observation period*

		Number of patients	
		Treated series	Control series
Mental disturbances		9	10
Aphasia		10	7
Cranial nerve signs		30	10
Dysarthria		1[illegible]	7
Motor function (muscle strength grade 0-5)	Grade 0	5	2
	1-3	12	3
	4-5	28	18
Cerebellar signs		3	8
Sensory signs		14	11
Reflex changes		36	37
Functional incapacity (0-100 %)	100 %	23	15
	75	1	4
	50 %	6	4
	25	3	8
	0	1	12
Epilepsy		5	5

Table 23. Data on the patients who survived the observation period while still under observation

	Treated series			Control series		
	Females	Males	Both sexes	Both sexes	Females	Males
No. of patients	14	18	32	32	12	20
No. of patients with hypertension	11	6	17	14	8	6
Mean age ± S.D. and range			64.1 ± 7.1 (43-75)	65.8 ± 7.4 (48-78)		
Degree of functional incapacity at the start of the study (No. of patients)		100 %	19	17		
		75 %	2	4		
		50 %	1	7		
		25 %	6	2		
		0	4	2		
Degree of functional incapacity at the end of the observation period (No. of patients)		100 %	13	9		
		75 %	0	3		
		50 %	6	4		
		25 %	3	7		
		0	10	9		

moderate preponderance of 100 per cent incapacitated individuals compared to the control' series.

Table 23 gives data on the 32 patients of both series who were alive and under observation at the completion of the study. Two additional patients were alive, namely one male of the treated series and one male of the control series, who had both been excluded because of myocardial infarction. The Table demonstrates the good conformity between the groups. A moderate improvement of the neurological status of both series had occurred during the observation period.

Fatalities

Clinical aspects

Of the total number of 100 patients, 34 died during the observation period, which averaged 3.2 years. Of these, 18 patients belonged to the treated series and 16 to the control series. Sixteen deaths in the treated series and 11 deaths in the control series occurred during the proper observation period.

Table 24 presents 6 patients who died during the anticoagulant period. In 5 of these the fatal outcome was more or less related to the cerebrovascular disorder. This gives a mortality rate of 4.3 per 1,000 observation-months.

Table 25 gives data on 6 patients who died during the placebo period. Patients Nos. 4 and 5 died of pneumonia after they had deteriorated because of recurrent *cerebral infarction* some months previously. It is reasonable to consider all these deaths as more or less related to the cerebrovascular disorder. This gives a mortality rate of 5.4 per 1,000 observation-months.

Thus there was an equal mortality rate of the two series during the anticoagulant and placebo period.

Table 26 presents data on the originally treated patients who died during the post-treatment period. Four of the 10 deaths were more or less related to the cerebrovascular

TABLE 24 *Patients who died during the period of anticoagulant therapy*

Sex and age (years)	Period of therapy (months)	Clinical cause of death	Autopsy findings: Recent or old thrombotic occlusion (t.o.) of cerebral vessels	Grade of atheromatosis (0-IV)	Recent or old cerebral infarction	Other findings
Male 63	16	Cerebral infarction	Old t.o of the internal carotid artery	IV	Old cerebral infarction	
Female 66	20	Cerebral hemorrhage	Not demonstrated	III	—·—	Fresh cerebral hemorrhage
Male, 62	9	—·—	—·—	III	Impossible to evaluate	—·—
Female 68	4	—·—	—·—	III	Old cerebral infarction	—·—
Female, 61	6	Operation for per forated gastric ulcer	—·—	II	—·—	Old cerebral hemorrhage
Male, 66	10	Enterocolitis and sepsis	—·—	I	—·—	

TABLE 25 *Patients who died during the period of placebo therapy*

Sex and age (years)	Period of placebo	Clinical cause of death	Autopsy findings: Recent or old thrombotic occlusion (t.o.) of cerebral vessels	Grade of atheromatosis (0-IV)	Recent or old cerebral infarction
Male, 51	15	Cerebral infarction	Recent and old t.o. of the internal carotid artery	III	Recent and old cerebral infarction
Male, 73	7	—·—	Not demonstrated	III	—·—
Male 76	19	—·—	—·—	III	Old cerebral infarction
Male 70	12	Pneumonia	Old t.o. of the internal carotid artery	III	—·—
Male, 70	13	—·—	Autopsy not performed		
Male 63	18	Sudden death	Autopsy not performed		

TABLE 26. *Patients who died after the completion of an ordinary anticoagulant period*

Sex and age (years)	Time after cessation of therapy (months)	Clinical cause of death	Autopsy findings: Recent or old thrombotic occlusion (t.o.) of cerebral vessels	Grade of atheromatosis (0–IV)	Recent or old cerebral infarction	Other findings
Male, 63	5	Diabetic coma and sepsis	Old t.o. of the internal carotid artery	I	Old cerebral infarction	
Male, 66	3/4	Sudden death	—	IV	—	Fresh coronary thrombus
Male, 67	7	Cerebral infarction	Recent and old t.o. of the internal carotid artery	II	Recent and old cerebral infarction	Recent small hemorrhages of cerebellum
Female, 68	9	Acute myocardial infarction	Old t.o. of the middle cerebral artery	III	Old cerebral infarction	Acute myocardial infarction
Female, 62	13	Congestive heart failure	Recent emboli(?) of the middle cerebral artery	II	Recent cerebral infarction	Pulmonary thrombo-embolism and an old myocardial infarction
Male, 70	6	Acute myocardial infarction	Not demonstrated	II	Old cerebral infarction	Acute myocardial infarction
Male, 69	26	Pneumonia and mental deterioration	—	III	—	
Male, 72	12	—	Autopsy not performed			
Male, 60	13	Sudden death	Autopsy not performed			
Female, 50	4	Acute myocardial infarction (verified by Ecg)	Autopsy not performed			

disorder (Patients Nos. 3, 7, 8, and 9). This gives a mortality rate of 5.4 per 1,000 observation-months.

Table 27 gives data on 5 patients of the control series who died after the completion of an ordinary placebo period. Four of these deaths had a probable relationship to the cerebrovascular disorder. This gives a mortality rate of 4.9 per 1,000 observation-months.

Thus during the post-treatment period there was no difference between the groups with regard to the mortality rate with a probable relationship to the cerebrovascular disorder.

Table 28 presents the 4 patients who died

TABLE 27 *Patients who died after the completion of an ordinary period of placebo therapy*

Sex and age (years)	Time after cessation of placebo (months)	Clinical cause of death	Autopsy findings: Recent or old thrombotic occlusion (t.o.) of cerebral vessels	Grade of atheromatosis (0–IV)	Recent or old cerebral infarction	Other findings
Female 78	20	Congestive heart failure	Not demonstrated	IV	Old cerebral infarction	Recent and old myocardial infarctions
Male 70	3	Pneumonia and mental deterioration	—•—	II	—•—	
Male, 69	19	—•—	Old t.o. of the internal carotid artery	III	—•—	Pulmonary thrombo-embolism and an old myocardial infarction
Male 60	[illegible]	—•—	Autopsy not performed			
Male, 67	10	—•—				

TABLE 28 *Patients who died after the placebo therapy had been discontinued earlier than intended*

Sex and age (years)	Time after cessation of placebo therapy (months)	Clinical cause of death	Autopsy findings: Recent or old thrombotic occlusion of cerebral vessels	Grade of atheromatosis (0–IV)	Recent or old cerebral infarction
Male, 69	3	Carcinoma of colon with metastases	Not demonstrated	III	Old cerebral infarction
Female, 77	7	Pneumonia and mental deterioration	Recent emboli of posterior cerebral artery	II	Recent and old cerebral infarctions
Female, 72	17	—•—	Autopsy not performed		
Male, 52	18	Sudden death			

after the placebo therapy had been discontinued earlier than intended.

There were 3 deaths in addition to the 31 cases presented in the Tables. Patients Nos. 6 and 7 of Table 12, who were excluded because of insufficient co-operability died of pneumonia 2 and 27 months after discontinuance of the therapy. Post-mortem examination was not made.

The third patient deserves some comments:

This was a 66-year-old moderately hypertensive male who was treated with placebo for 25 months. Four months after the termination of this regime he experienced an acute myocardial infarction and long-term anticoagulant therapy was instituted. After another 11 months a fatal cerebral hemorrhage occurred at a T.T. level of 14 per cent. At autopsy a massive fresh cerebral hemorrhage was found. There were grade IV atheromatous lesions of the cerebral arteries. The most important finding was a cystic lesion with a diameter of 3 cm, presumably an old hemorrhage. This was assumed to be the original cerebral lesion. Thus, this patient was included in the study because of a faulty diagnosis. There was nothing to suggest that this lesion contributed to the final outcome. The fatal accident occurred after the patient had been excluded from observation according to rules settled prior to the study. This complication is therefore not included in the figures.

Autopsy findings

In 23 cases or 68 per cent of the 34 dead patients post-mortem examination was made. The relatively low autopsy rate reflects the difficulties in obtaining a post-mortem examination when death occurred outside hospital. Of the 12 patients who died during the anticoagulant and placebo period 10 were autopsied. This was done in 10 of the 15 patients who died during the post-treatment period.

A regular post-mortem examination was made including a close observation of the extracranial and intracranial portion of the cerebral arteries. However the extracranial part of the vertebral arteries was not examined as a matter of routine. The brain was thereafter fixed for subsequent macroscopical and microscopical examination by a neuropathologist, who had no knowledge of the treatment groups.

The most important findings are summarized in Tables 24 to 28. The degree of atheromatosis was classified in accordance with an International Coding Guide (World Federation of Neurology 1959).

The first case in Table 24 was the only death from a recurrent cerebral infarction which occurred during the anticoagulant therapy. During the relevant period an adequate hypocoagulability of blood was achieved (Table 15). Although there was clear-cut clinical evidence of a recurrent cerebral stroke neither a fresh thrombotic occlusion nor a recent cerebral infarction was demonstrated. However there were prominent atheromatous lesions of the cerebral arteries.

According to clinical criteria, 5 of the cases presented in Tables 24 to 26 developed a recurrent cerebral infarction during the period immediately prior to death. In only 2 of these was a recent thrombotic occlusion demonstrated. In 2 of the cases the pathologist was unable to demonstrate a recent cerebral infarction. Probably death occurred before microscopical changes of the cerebral tissue had developed.

From the Tables it is evident that the original clinical diagnosis of cerebral infarction was confirmed in the majority of the cases. Not so encouraging from an anticoagulant point of view was the fact that an old thrombotic occlusion of cerebral vessels was demonstrated in less than half of the cases. A microscopical examination of the cerebral vessels was not performed. Even if this is made it might be extremely difficult to differentiate between advanced atheromatous arterial lesions and old thrombotic processes. In addition the extracranial portion

of the vertebral arteries was not routinely examined. The possibility also exists that vessels occluded by thrombotic occlusions may be recanalized. It is tempting to suggest such an explanation in cases like the last patient of Table 24 in whom a medium-sized cerebral infarction was demonstrated, although no occlusions of cerebral vessels were found and there were only slight atheromatous arterial changes.

One patient has been presented in some detail in whom autopsy proved the original lesion to have been a hemorrhage. In addition Table 24 presents data on one patient in whom the post mortem examination revealed both an old cerebral infarction and an old cerebral hemorrhage. Thus, although reasonable precautions were taken, at least 2 patients of the present study were included because of a wrong diagnosis. In neither of them, however was it found likely that the original cerebral hemorrhage contributed to the fatal outcome.

In 2 of the 3 fatal cases of a cerebral hemorrhage, autopsy proved the original lesion to have been a cerebral infarction. In the last case the massive destruction of the brain by the fresh bleeding made such an evaluation impossible.

Chapter V

Discussion

The first chapters were concerned with methodological, diagnostical, and terminological problems. Here comments are confined to the results obtained.

We want to emphasize the importance of using the double blind technique in a study of this kind. In our disabled patients it was often difficult to evaluate the great variety of individual sensations versus the neurological signs. We consider it a great benefit that the neurologist and the pathologists were unaware of the distribution of patients.

It is important to decide if the two series of cases were comparable and of a sufficient size. Beforehand it was debated whether adjustments should be made for factors of particular prognostic significance. Among these age and hypertension are well known (Marshall & Kaeser 1961) The sex, over weight, the size and localization of the cerebral damage, the angiographic findings, and the degree of functional incapacity may also be prognostic factors. Theoretically therefore a series of clinical features should deliberately have been evenly distributed between the groups Among these it was found difficult to differentiate one or two factors for which it might have been possible to arrange subadjustments. Instead we hoped that the study would comprise a sufficiently large number of cases to ensure an even distribution of important factors by chance. The distribution of such factors is demonstrated in Tables 5 to 10 Great similarities between the series existed, although minor deviations are also noted. The treated series comprised a larger number of females and

also of hypertensive females. But the moderate number of severely hypertensive individuals was evenly distributed. In the treated males the mean age and the mean serum cholesterol level were somewhat lower than the corresponding values of the control series. On the other hand, there were more patients in the treated series with particularly high serum cholesterol levels whereas the control series comprised a larger number of individuals with over weight. In the control series there were a few more patients who had experienced more than one cerebral accident. In the control series 18 patients had normal cerebral angiography while there were 13 such cases in the treated series. In the treated series there was moderate accumulation of 100 per cent incapacitated individuals and of those with a motor function of grade 0. None of these differences are of statistical significance and the similarities between the groups are far more marked.

When considered separately the two series are probably too small to permit conclusions of prognostic factors. The correctness of treating patients from the two series together may be questionable. If this is done the following data are obtained from Tables 5, 8, 10 14 and 23. Of the initial number of 38 females 26 were still under observation at the end of the study. The corresponding figures for the males were 62 and 38. Males predominated among the patients who experienced a recurrent cerebral infarction during the treatment period. Thus the treated series had more favourable sex ratio than the control series. Of 52 hypertensive individuals, 31 were still under observation at the end of the study whereas the corresponding figures for non-hypertensive individuals were 48 and 33 respectively. Of 59 patients who were 100 per cent incapacitated initially 35 were under observation at the end of the study. Of 41 patients who had not been totally incapacitated, 31 were under observation at the end. Four of the 5 patients of the treated series who had a recurrence during the first part of the study were hypertensive while two of them were initially 100 per cent incapacitated. Of the corresponding 10 patients of the control series 5 were hypertensive while 9 had been 100 per cent incapacitated at the start. As might be expected, therefore, hypertension, and especially the degree of functional incapacity seem to be factors of poor prognostic significance. Probably owing to the limited number of patients this could not be proved by statistical methods. However the moderate accumulation in the treated series of factors which might imply an unfavourable prognosis should be borne in mind when the results are evaluated. On the other hand, this series had a more favourable sex ratio than the controls. It should also be realized that the above-mentioned data do not necessarily imply that a moderate accumulation of these factors in one series *is fact* influenced the clinical course during the anticoagulant or placebo period.

These points illustrate the importance of making careful evaluation of the two groups of patients in a clinical trial and of presenting a detailed documentation of their comparability. Although no differences of statistical significance are demonstrated by comparison of individual clinical features, the possibility exists that by an additive effect a definite accumulation of poor prognostic factors may occur in one series. Especially if only a limited number of patients is available may such a situation appear and influence the results.

The comparability of the groups and thus the validity of the present study might have been increased if adjustments had been made for sex, hypertension, and the degree of functional capacity. Nevertheless we believe that the two series had a sufficient degree of comparability to fulfil the requirements of a clinical trial.

From Table 11 and Figures 1 to 3 it is evident that the two series differed with respect to the hypocoagulability of blood caused by the anticoagulant treatment. If the total number of T.T. determinations is used in such a presentation there will be an over-representation of less satisfactory values i.e. values outside the intended range. It was found more useful to calculate the approximate single weekly T.T. per cent of each patient during the treatment period. This was done by using the average value of succeeding T.T. determinations as representative of the intervening period. Even this method is far from ideal. Especially is this true of periods immediately after changes of the anticoagulant dose. In our opinion, however this method gives a fairly adequate picture of the treatment.

It was decided to maintain a relatively intense anticoagulant therapy. The intended range with a T.T. value between 10 and 25 per cent necessarily implied periods with T.T. levels below 10 per cent. Such values were obtained during 8.9 per cent of the total treatment period (Figure 2). Therefore, the possibility of producing a cerebral hemorrhage as a complication of the treatment was felt as a constant menace throughout the study. It might be argued that the grave significance and the increased liability of such a complication should have warranted a less intensive anticoagulant program. However we thought it unwise to study the effect of an anticoagulant regime without making use of a dosage scheme from which an optimal degree of antithrombotic effect might be expected.

We are unaware of other studies in the cerebral field with a similar documentation of the anticoagulant therapy. Therefore, the results have been compared with studies in the cardiac field, and those of Bjerkelund (1957) and Borchgrevink (1960) were chosen. These were concerned with the long-term effect of anticoagulants on patients with a myocardial infarction or with angina pectoris. In the present study the T.T. values were below *25 per cent* during *86 per cent* of the total treatment period. The P & P values of Bjerkelund's study were less than *30 per cent* during *82 per cent* of the treatment period, whereas the corresponding value in Borchgrevink's study was *93 to 94 per cent*. In the present study the grand mean of the weekly T.T. per cents was 18.6 with a standard deviation of 4.0 whereas the corresponding value for the *total number* of P & P determinations in Borchgrevink's study was 19 per cent with a standard deviation of 4.3. Borchgrevink's patients were treated somewhat more intensively than those of Bjerkelund. In the present study T.T. and not P & P determinations were performed. It has been shown, however that with the thromboplastin used in this country this difference is without significance (Owren 1959). In the 3 studies somewhat different methods for the presentation of anticoagulant data were used. These differences are of minor importance. It may be safely concluded that the anticoagulant therapy of the present study was performed with an intensity well comparable to these studies and probably in closer accordance with that of Borchgrevink than with that of Bjerkelund.

In both of these studies a protective effect from the long-term anticoagulant therapy was demonstrated. Theoretically therefore, the intensity of the anticoagulant regime of the present study provided a blood hypocoagulability of antithrombotic significance. On the other hand, there is little to suggest that the treatment was too intensively performed and included an unacceptable danger of hemorrhagic complications.

Tables 12 and 13 present data on patients in whom the anticoagulant and placebo treatment were discontinued earlier than intended. Apart from the patients who died of a cerebral cause, no particular differences

between the two groups are noted. The exclusion of these patients did not influence the comparability of the remaining patients.

During the treatment period the two series showed similar mortality rates (Tables 24 and 25). This result was unaffected whether all deaths or only those with a probable relationship to the cerebrovascular disorder were considered. During the post-treatment period, however, 10 deaths occurred in the originally treated series as against 5 among the controls (Tables 26 and 27). The difference was mainly related to the occurrence of several cases of fatal myocardial infarctions in the treated series. However, if only deaths with probable relationship to the cerebrovascular disorder were compared, similar mortality rates were obtained. During the total of 319.8 patient-years 16 deaths occurred in the treated series and 11 among the controls. The mortality rate of the total case material during the complete observation period was 8.4 per 100 observation-years. The corresponding value in the study by Thygesen *et al.* (1964) was 6.3.

During the first part of the study 5 patients of the treated series suffered a new cerebral infarction, against 10 of the control series. The recurrence rate per 1,000 observation-months was 4.3 for the treated series and 10.0 for the control series. If the usual 5 per cent level is used as the critical limit these differences are without statistical significance.

When these figures are evaluated the liability of recurrent cerebral infarction to occur among males should be recalled (Table 14). Compared to the control series there was predominance of females in the treated series (Table 5). From a prognostic point of view the treated series had a more favourable sex ratio than the control series. On the other hand, the latter group contained a greater number of less disabled individuals than the treated series.

The post-treatment period for the originally treated and control patients averaged 14.7 and 16.7 months respectively. During this period the recurrence rate of the "treated" series was 6.6. per 1,000 observation-months compared to 6.1 of the "control" series. This suggests that the clinical courses of the two series were more equal after the cessation of the anticoagulant therapy than before.

It is impossible to decide whether these results were obtained by chance or whether they indicate a moderate beneficial effect from long-term anticoagulant therapy which might have been demonstrated beyond statistical doubt if a greater number of patients had been available. However, additional aspects should be considered before conclusions are drawn.

Table 15 shows that a new cerebral infarction or T.I.A. in all instances occurred during periods with a satisfactory hypocoagulability of blood as indicated by the T.T. per cent. It may be noted that the patients who suffered a cerebral recurrence were treated during such periods according to the intended scheme, i.e. that these episodes were scarcely related to a failure of therapy. But there are also discouraging aspects. In these patients an adequate anticoagulant regime failed to prevent the development of a recurrent cerebral infarction. This fact has close bearing on problems discussed in chapter I.

The so-called therapeutic range, comprising T.T. values from 10 to 20 or 25 per cent, is rather diffuse conception, which is mainly determined by experience. The intention is to obtain a reasonable hypocoagulability of blood without involving an undue risk of hemorrhagic complications. The T.T. level is only one among a series of factors which might influence the thrombotic tendency on an actual arterial site in the individual patient. The hypocoagulability of blood produced by peroral anticoagulants can only exert an effect on the

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It was decided to maintain a relatively intense anticoagulant therapy The intended range with a T T value between 10 and 25 per cent necessarily implied periods with T T levels below 10 per cent. Such values were obtained during 8.9 per cent of the total treatment period (Figure 2) Therefore, the possibility of producing a cerebral hemorrhage as a complication of the treatment was felt as a constant menace throughout the study It might be argued that the grave significance and the increased liability of such a complication should have warranted a less intensive anticoagulant program. However we thought it unwise to study the effect of an anticoagulant regime without making use of a dosage scheme from which an optimal degree of antithrombotic effect might be expected.

We are unaware of other studies in the cerebral field with a similar documentation of the anticoagulant therapy Therefore, the results have been compared with studies in the cardiac field, and those of Bjerkelund (1957) and Borchgrevink (1960) were chosen. These were concerned with the long-term effect of anticoagulants on patients with a myocardial infarction or with angina pectoris. In the present study the T T values were below 25 per cent during 86 per cent of the total treatment period. The P & P values of Bjerkelund's study were less than 30 per cent during 82 per cent of the treatment period, whereas the corresponding value in Borchgrevink's study was 93 to 94 per cent In the present study the grand mean of the weekly T T per cents was 18.6 with a standard deviation of 4 0 whereas the corresponding value for the total number of P & P determinations in Borchgrevink's study was 19 per cent with a standard deviation of 4.3 Borchgrevink's patients were treated somewhat more intensively than those of Bjerkelund. In the present study T T and not P & P determinations were performed. It has been shown, however that with the thromboplastin used in this country this difference is without significance (Owren 1959) In the 3 studies somewhat different methods for the presentation of anticoagulant data were used. These differences are of minor importance. It may be safely concluded that the anticoagulant therapy of the present study was performed with an intensity well comparable to these studies and probably in closer accordance with that of Borchgrevink than with that of Bjerkelund.

In both of these studies a protective effect from the long-term anticoagulant therapy was demonstrated. Theoretically therefore, the intensity of the anticoagulant regime of the present study provided a blood hypocoagulability of antithrombotic significance. On the other hand, there is little to suggest that the treatment was too intensively performed and included an unacceptable danger of hemorrhagic complications.

Tables 12 and 13 present data on patients in whom the anticoagulant and placebo treatment were discontinued earlier than intended. Apart from the patients who died of a cerebral cause, no particular differences

may such lesions give rise to small perivascular bleedings. The role played by anticoagulants is probably to aggravate a bleeding which otherwise might have been limited and without clinical importance.

With the intended range of blood hypocoagulability it was impossible to avoid transitory T T values below 10 per cent, i.e. periods during which a particular danger of hemorrhagic complications existed. In one of the above-mentioned patients a T T value of 9 per cent was recorded at the time of the episode, whereas the other two had percentages of 16 and 27 respectively.

It seems justified to conclude that some few cases of cerebral hemorrhage are inevitable complications during long-term anticoagulant treatment of patients with a cerebral infarction, and particularly in those with an elevated blood pressure. Evidently this is the case even when the thrombotest method is used for blood control.

The difference between three such complications in one group and none in the other is of no statistical significance. However for reasons discussed below we feel that such fatalities have a particular significance unrelated to statistical considerations.

This study was not designed to evaluate the preventive effect from anticoagulants on T.I.A.s. However this point is of particular interest because it has been repeatedly maintained that anticoagulants have such an effect. One patient only of the treated series and two of the control series were included in the study because of such attacks. None of these had new cerebral attack. During the treatment period a total of 4 T.I.A.s occurred in the treated series against 10 in the control series. Although this difference is without statistical significance a moderate preventive effect from anticoagulants is suggested which is not present after cessation of therapy. Such attacks have well-known tendency to recur in some patients. From a statistical point of view it is more correct to compare the number of patients involved and not the total number of attacks. If this is done no particular difference is noted between the small number of patients in both series. Finally it is evident from Table 15 that the T.I.A.s of the treated series in all instances occurred during periods with T T values within the intended range. If the suggestion made by Gunning *et al.* (1964) is correct, namely that such attacks are often caused by small platelet emboli originating from atheromatous arterial lesions, no preventive effect from anticoagulants would be expected.

During the treatment period there was a total number of 9 recurrent cerebral infarctions and T.I.A.s in the treated series against 21 of the control series. This uneven distribution is statistically significant. However for various methodological reasons such a combination of results may be considered questionable.

During the treatment period an acute myocardial infarction occurred in two patients of the control series and in one patient of the treated series. In the latter case the infarction developed during a period with T T values within the intended range (Table 15). During the post-treatment period there were 4 fatal cases of myocardial infarction in the originally treated series against one in the control series. During the total observation period there were in the total case material 5 cases of cerebral infarction and 5 of myocardial infarction which were all fatal. Thus the grave cardiac prognosis of patients with cerebral infarction is demonstrated.

There are some direct and indirect clinical observations to indicate an increased risk of thrombo-embolic manifestations in the immediate period after the termination of anticoagulant therapy (Carter *et al.* 1958, Dixon *et al.* 1960, Poller & Thomson 1964). This is probably related to a rebound response, i.e., a state of hypercoagulability related to the cessation of the drug effect on the production of coagulation factors in the liver. As a con-

development of the red thrombus secondary to the initial white thrombus formed by aggregates of platelets. When formed upon a pre-existing atheromatous plaque it is possible that such a white thrombus alone may lead to circulatory disturbances and to the development of a cerebral infarction. In addition other pathogenetic mechanisms discussed previously may be responsible for the development of a cerebral infarction.

The same criteria were followed in the diagnosis of a recurrence as of the initial lesion, with the exception that a cerebral angiography was usually omitted. Except for one of the T.I.A.s indicated in Table 15 no obvious extracerebral pathogenetic mechanisms were disclosed as a cause of a recurrence. At autopsy the correctness of the original clinical diagnosis was confirmed in the great majority of cases (Tables 24 to 28). However in less than half of the cases an old thrombotic occlusion of the relevant cerebral vessels was demonstrated. The degree of atheromatous arterial lesions also showed considerable variation. Thus the limitations, discussed previously, of the post-mortem examination in cerebrovascular disease are emphasized.

Data on the 11 patients who suffered a complication during the phenylindandione therapy are presented in Table 17. One patient had a severe allergic dermatitis, while the other 10 experienced bleeding episodes, 4 of which were serious and 3 fatal. The frequency of bleeding complications was one hemorrhage for every 9.7 treatment years. The corresponding values in Bjerkelund's study (1957) and Borchgrevink's (1960) were 13.1 and 14.2 respectively. In the large study on anticoagulants in cerebrovascular disease performed by Baker *et al.* (1962) it may be calculated that one bleeding occurred for every 2.8 treatment years. This high frequency was probably related to the co-operative approach. In the study by Thygesen *et al.* (1964) who made use of the P & P method for blood control, three cerebral hemorrhages occurred in the treated series.

It is important whether the fatal complications might have been prevented. The diagnostic methods included a lumbar puncture and a cerebral angiography. In two of these cases there was nothing at autopsy to suggest that the original cerebral lesion had been a bleeding. In the last patient this question could not be decided. However despite the above-mentioned precautions autopsy showed that the original cerebral lesion in at least two cases had been a cerebral hemorrhage. But in neither of them was it found likely that the lesion contributed to the fatal outcome.

The particular risk of inducing a cerebral hemorrhage during anticoagulant therapy in hypertensive patients was already indicated in the study of Bjerkelund (1957) and further emphasized in that of Bradford Hill *et al.* (1960). The 3 patients of the present study who had a cerebral hemorrhage were all moderately hypertensive with a diastolic blood pressure below 120 mm Hg and hypertensive retinal changes of grade 2. None of them were treated with hypotensive drugs. Two were initially 100 per cent and the last 50 per cent incapacitated. At the time of the final accident they were all making a satisfactory recovery and one of them had resumed his ordinary occupation. Therefore in these patients, there was nothing to suggest a particularly grave prognosis. Hypertension is such a common clinical finding in patients with a cerebral infarction that it would be very difficult to collect a sufficiently large case material of normotensive individuals. Such a study would only have a limited general interest.

It is reasonable to relate the increased risk of a cerebral hemorrhage during anticoagulant therapy to pre-existing arterial lesions of a thrombotic and/or an atheromatous nature. Especially in hypertensive individuals

attacks occurred per 100 observation years. Again, a definite difference from the study by Thygesen et al. (1964) is noted. The average observation period of their study was about 20 months, and the rate of transient ischemic attacks in the total case material was 18.2 per 100 observation years.

Until recent years knowledge regarding the natural history of cerebrovascular diseases has been inadequate. The selected character of the various case materials concerned with the use of anticoagulant therapy should be kept in mind. This fact does not reduce the importance of the present results, which support others in this field, namely that patients with cerebral infarction have more favourable prognosis than usually expected.

It may be questioned whether the number of patients was sufficient for the purpose of the study. Undoubtedly a greater number of patients would have been desirable. W feel that our conclusions are so well founded that it seems unlikely that a greater number of patients would have given another result. Furthermore, the size of the present case material is well comparable with most studies in this field. The largest of them is the co-operative study reported by Baker et al. (1962) which comprised 132 patients with cerebral thrombosis

In chapter II it was concluded that an evaluation of the literature gave a slight advantage to the assumption that anticoagulants are without value in the long-term treatment of patients with a cerebral infarction. This conclusion is substantiated by the present study. This does not mean that we exclude slight protective effect from anticoagulants in cerebrovascular disease. In fact the present study might suggest such possibility. However our conclusions are influenced by the following points:

The so-called therapeutic range of anticoagulant therapy has only an uncertain relationship to a direct antithrombotic effect. We believe that the development of a probable cerebral infarction in several patients during periods with a blood hypocoagulability within the intended range also has a bearing on the varied pathogenetic mechanisms of cerebral infarction. This is the reason why a potential preventive effect from anticoagulants would require a considerably larger case material in order to become established.

The present study suggests an increased risk of thrombotic manifestations during the first months after cessation of anticoagulants. This might be considered a restrictive factor for their use unless life-long therapy is intended.

Finally our conclusions are decisively related to the danger of inducing a cerebral hemorrhage. The data on the anticoagulant regime and the frequency of bleedings do not suggest that too intensive an anticoagulant regime was performed. Nevertheless, there were 3 fatal cases of a cerebral hemorrhage. These occurred despite reasonable precautions taken prior to the institution of therapy. The results of other studies in addition to our own clearly indicate that cerebral hemorrhages are inevitable consequences of long-term anticoagulant therapy in this category of patients. Although no difference of statistical significance was demonstrated, the occurrence of these deaths can hardly be overestimated. We believe that such experiences are something more than ordinary complications. From an ethical point of view it might be considered unacceptable to counterbalance the *actual occurrence* of a fatal cerebral hemorrhage with the *theoretical prevention* of a fatal cerebral infarction. It is reasonable to favour this opinion because the former fatality is so directly related to a therapy without which the patient might still have enjoyed life. Indeed, nil nocere is a motto of medical ethics.

Our conclusion is that the present study

sequence it is widely maintained that such therapy should be tapered off gradually Marshall (1963) found that the incidence of cerebrovascular accidents increased during the first 3 months after anticoagulants were withdrawn from patients who had previously had a completed stroke Recently Marshall & Reynolds (1965) demonstrated that after gradual withdrawal of anticoagulant therapy from patients who had previosly had transient ischemic attacks there was an increased frequency of such episodes compared to an adequate control group. However each group consisted of only 13 patients.

The present study was used for similar observations. In addition, the anticoagulant therapy was tapered off gradually in every other patient. One death from an acute coronary occlusion occurred 3 weeks after the abrupt discontinuance of anticoagulant therapy In the remaining patients who suffered a cerebral episode or an acute myocardial infarction the therapy was terminated gradually Thus, there was little to suggest that the method of terminating the therapy influenced the clinical course.

Apart from one case of fatal myocardial infarction, there were no other episodes of a probable thrombotic origin in the treated series during the first 3 months after cessation of therapy In the control series there were 2 non-fatal cases of a recurrent cerebral infarction. During the next post-treatment months there were some cases of fatal myocardial infarctions in the treated series. Although these occurred a relatively long time after the cessation of therapy it cannot be excluded that the discontinuance of anticoagulant therapy might involve a risk of inducing a myocardial infarction in this group of patients.

The relatively favourable prognosis of both series of patients should be emphasized. This was a selected case material. Neverthe less, it might be considered encouraging that two-thirds of the patients were alive at the end of an observation period which averaged more than 3 years. One-third of these patients were 100 per cent incapacitated, while two-thirds of the original case material consisted of such individuals. This difference mainly reflects the poor prognosis of those totally incapacitated. But the prognostic aspects may also be presented in following way among the 64 patients who were still under observation 36 patients had originally been 100 per cent incapacitated. At the end there were 22 such cases.

When Tables 10 16, 22, and 23 are compared, a moderate improvement of the neurological status of both groups throughout the study is noted. There was nothing to suggest a more favourable neurological rehabilitation of the treated series during the anticoagulant period.

The low frequency of recurrent cerebral infarctions of both series throughout the study is also remarkable. A total of 26 recurrences occurred in 24 of the 100 patients during an average observation period of 3.2 years, i.e. a recurrence rate of 8.1 per 100 observation years. If the number of cerebral hemorrhages is also included, the rate of cerebrovascular accidents of the total case material was 9 0 per 100 observation years, while the corresponding value in the study by Thygesen *et al.* (1964) was 14.2. Another method of selection of cases might be the explanation of this difference. However the over all mortality rate of the present study was 8 4 per 100 observation years against 6.3 in the Danish study Thus, there was nothing to suggest that the Danish material originally consisted of patients with a less favourable prognosis. This clearly demonstrates how difficult it is to compare case materials from different places. The rate of cerebrovascular accidents of the present study corresponded to that of Bradford Hill *et al.* (1962)

During the total period of study 23 T.I.A.s occurred in 16 patients. Thus, 7 1 such

emboli; those who were severely hyper tensive; those who were found unable to attend regularly for control of the anticoagulant therapy on an out-patient basis; and finally those in whom a possible extracerebral pathogenetic mechanism of the cerebrovascular accident was disclosed. Thus

relatively homogeneous case material remained, presumably consisting of patients with a cerebral infarction caused by thrombotic and/or atheromatous arterial lesions. These were allocated to a treated and a control series according to a preconstructed random list. The treated series was given phenylindandione with the aim of inducing a hypocoagulability of blood between 10 and 25 per cent as measured by the thrombotest method. During the hospital course, T T determinations were made twice weekly. Later these were done at intervals of 3 to 5 weeks or more frequently when required. The patients of the control series were given placebo and submitted to the same clinical and laboratory control as the treated patients. Throughout the study the double blind technique was followed. The neurologist examined the patients at intervals of 6 months, or more frequently when required. A total of 100 patients were given anticoagulant or placebo therapy for at least a two-year period and were then followed for at least 6 months. The study lasted 5 years. The anticoagulant therapy was terminated abruptly in every other patient and tapered off gradually over a 3-week period in the remainder.

The treated series consisted of 51 patients in whom the average treatment and post treatment periods lasted 22.8 and 14.7 months respectively. The corresponding values for the 49 patients of the control series were 22.6 and 16.7 months. General medical and neurological features of the two series were compared: the age and sex composition, the serum cholesterol level, the presence of hypertension and over weight, the period from the acute episode until inclusion in the study, the clinical localization of the cerebral insult, the neurological defects and the degree of functional incapacity and finally the results of the electroencephalography and of the cerebral angiography.

The two series were found comparable in all important respects except for a slight accumulation in the treated series of hyper tensive females and of 100 per cent incapacitated individuals, whereas there was a moderate predominance of males in the control series.

Chapter IV presents the results. To demonstrate that the two series differed with respect to the hypocoagulability of blood a detailed documentation on this point is given. The grand mean of the mean weekly T T percentages was 18.6 per cent. The T T values were above 25 per cent during 14 per cent of the total anticoagulant period of 1 162 patient-months and below 10 per cent during 8.9 per cent of it. These results are comparable to those of the studies in the cardiac field in which a preventive effect from anticoagulants has been demonstrated.

During the treatment period there were 6 deaths in both groups. Five of the deaths in the treated series and all of those in the control series were related to the cerebrovascular disorder. The corresponding mortality rates per 1,000 observation months were 4.3 and 5.4 of the treated and control series respectively. During the post-treatment period 10 deaths occurred in the originally treated series against 5 deaths among the controls. The difference was mainly related to several cases of fatal myocardial infarction in the "treated" series. When only deaths with probable relationship to the cerebrovascular disease were considered, the mortality rate per 1,000 observation months was 5.3 for the "treated" patients and 4.9 for the controls. Thus, during the two parts of the study there was no difference between the

speaks against the long-term use of peroral anticoagulants in the treatment of patients with a completed cerebral infarction of a *probable thrombotic* and/or atheromatous origin

This conclusion would not have been so confident without *these cerebral hemorrhages*, which probably not had occurred if only normotensive individuals had been included. In our opinion the effect of long-term anticoagulant therapy in normotensive patients with a cerebral infarction has not yet been finally decided. However it would be extremely difficult to collect a sufficiently large case material of normotensive individuals and such a study would only be of limited general importance.

It is unlikely that similar research in the anticoagulant field will provide advances in the treatment of patients with cerebral infarction An increased knowledge of the basic processes involved in the development of atheromatosis and thrombosis is the key to progress.

Chapter VI

Summary

This study was undertaken to evaluate the effect of long-term peroral anticoagulant therapy in patients with a cerebral *infarction* of a presumable thrombotic and/or atheromatous origin. During a controlled clinical trial following the double blind technique, two series of randomized patients were used for observations of the mortality rate, the recurrence rate, and of complications related to *anticoagulant therapy*

Chapter I is concerned with problems related to the study of anticoagulants in cerebrovascular disease. Clinical and pathological aspects of the diagnosis *and the* terminology of cerebrovascular disorders are reviewed. The variety of pathogenetic mechanisms which may be involved during the development of a cerebral infarction is emphasized. Theoretical and practical problems related to the use of anticoagulants in cerebral infarction are *discussed*.

Chapter II presents a survey of the literature. Some recent studies were selected for review and *evaluation. Few of these were found to* fulfil the requirements for a controlled clinical trial. Reports providing evidence both for and against a protective effect from anticoagulants in patients with a cerebral infarction exist. A careful evaluation seems to give a slight preference to the assumption that anticoagulants are without value in the long-term treatment of these patients.

Chapter III presents the methods of study and the case material. Patients below 75 years of age *admitted to hospital* because of a recent cerebrovascular accident were submitted to medical and neurological examination, including a lumbar puncture, cerebral angiography and encephalography The following categories *of patients were* excluded. patients suspected of a cerebral hemorrhage or of a cerebral infarction caused by

emboli; those who were severely hypertensive; those who were found unable to attend regularly for control of the anticoagulant therapy on an out-patient basis; and finally those in whom a possible extracerebral pathogenetic mechanism of the cerebrovascular accident was disclosed. Thus

relatively homogeneous case material remained, presumably consisting of patients with a cerebral infarction caused by thrombotic and/or atheromatous arterial lesions. These were allocated to a treated and a control series according to a preconstructed random list. The treated series was given phenylindandione with the aim of reducing a hypocoagulability of blood between 10 and 25 per cent as measured by the thrombotest method. During the hospital course, T T determinations were made twice weekly. Later these were done at intervals of 3 to 5 weeks or more frequently when required. The patients of the control series were given a placebo and submitted to the same clinical and laboratory control as the treated patients. Throughout the study the double blind technique was followed. The neurologist examined the patients at intervals of 6 months, or more frequently when required. A total of 100 patients were given anticoagulant or placebo therapy for at least a two-year period and were then followed for at least 6 months. The study lasted 5 years. The anticoagulant therapy was terminated abruptly in every other patient and tapered off gradually over 3-week period in the remainder.

The treated series consisted of 51 patients in whom the average treatment and post treatment periods lasted 22.8 and 14.7 months respectively. The corresponding values for the 49 patients of the control series were 22.6 and 16.7 months. General medical and neurological features of the two series were compared: the age and sex composition, the serum cholesterol level, the presence of hypertension and over-weight, the period from the acute episode until inclusion in the study, the clinical localization of the cerebral insult, the neurological defects and the degree of functional incapacity and finally the result of the electroencephalography and of the cerebral angiography.

The two series were found comparable in all important respects except for a slight accumulation in the treated series of hypertensive females and of 100 per cent incapacitated individuals whereas there was a moderate predominance of males in the control series.

Chapter IV presents the results. To demonstrate that the two series differed with respect to the hypocoagulability of blood a detailed documentation on this point is given. The grand mean of the mean weekly T T percentages was 18.6 per cent. The T T values were above 25 per cent during 14 per cent of the total anticoagulant period of 1 162 patient-months and below 10 per cent during 8.9 per cent of it. These results are comparable to those of the studies in the cardiac field in which a preventive effect from anticoagulants has been demonstrated.

During the treatment period there were 6 deaths in both groups. Five of the deaths in the treated series and all of those in the control series were related to the cerebrovascular disorder. The corresponding mortality rates per 1,000 observation months were 4.3 and 5.4 of the treated and control series respectively. During the post-treatment period 10 deaths occurred in the originally treated series against 5 deaths among the controls. The difference was mainly related to several cases of fatal myocardial infarction in the "treated" series. When only deaths with a probable relationship to the cerebrovascular disease were considered, the mortality rate per 1,000 observation months was 5.3 for the "treated" patients and 4.9 for the controls. Thus, during the two parts of the study there was no difference between the

groups in regard to the mortality rate with probable relationship to the cerebrovascular disorder

During the treatment period there was one fatal case of a recurrent cerebral infarction in the anticoagulant group against 3 such cases in the placebo group. A total of 5 patients in the treated series had a recurrent cerebral infarction compared to 11 such events in 10 patients of the control series. The recurrence rate per 1 000 observation months was 4.3 and 10.0 for the treated and control series respectively. The difference between the number of cases in the two groups who had a recurrent cerebral infarction is not of statistical significance ($p = 0.23$). Four patients in the treated series had a total of 4 T.I.A.s, whereas 10 such episodes occurred in 6 patients of the control series. There was a total of 9 non-hemorrhagic cerebral episodes in the treated series compared to 21 in the control series. This difference is statistically significant ($p = 0.024$). However such a combination of results is a questionable procedure.

In all instances the cerebral infarctions and the T.I.A.s of the treated series occurred during periods with T.T. values within the intended range.

Ten hemorrhagic complications or one per 9.7 treatment years were noted. Of these 4 were serious and 3 fatal. The 3 patients who had a fatal cerebral hemorrhage were all moderately hypertensive. In one case the T.T. value was 9 per cent at the time of the bleeding.

At the end of the observation period no significant differences between the neurological status of the patients of the two series were noted.

During the post-treatment period there was one fatal case of a recurrent cerebral infarction in the treated series against none of the control series. Five cerebral infarctions occurred in 4 patients of the treated series compared to 5 such episodes in 5 patients of the control series. The recurrence rate per 1 000 observation months was 6.6 and 6.1 for the treated and control series respectively. During the post-treatment period 4 patients of the treated series experienced a total of 7 T.I.A.s whereas 2 such attacks occurred in 2 patients of the control series.

During the treatment period there was one non-fatal case of an acute myocardial infarction in the treated series, whereas two such events occurred among the placebo patients. During the post-treatment period 4 patients of the treated series had a fatal myocardial infarction, whereas this happened in one patient of the control series. During the first 3 post-treatment months there was no case of a recurrent cerebral infarction in the treated series whereas two such episodes occurred in the control series. However in the treated series there was one death from an acute coronary occlusion 3 weeks after the anticoagulant therapy had been terminated abruptly. Apart from this case there was nothing to suggest that the mode of terminating the anticoagulant treatment influenced the clinical course. The possible danger associated with the conclusion of such a therapy was hinted at by the moderate accumulation in the treated series of fatal myocardial infarctions during the first months of the post-treatment period.

At the end of the total observation period, which averaged 3.2 years, 32 patients of both series were alive and under observation. In both series a moderate improvement of the neurological status had occurred. No significant differences between the groups were noted. A total of 34 patients had died. According to rules settled prior to the study 27 of these deaths occurred during the proper observation period. Of these, 16 patients belonged to the treated series and 11 to the controls. Twenty-three of the 34 patients were submitted to a post-mortem examination, including a neuropathological study. In the

great majority of the cases the original clinical diagnosis of a cerebral infarction was confirmed. In the only patient who died of a recurrent cerebral infarction during anticoagulant therapy neither a recent thrombotic occlusion nor a new cerebral infarction was demonstrated. However extensive atheromatous arterial lesions and an old cerebral infarction were present. During the total observation period there were in all 5 fatal recurrent cerebral infarctions in the two series. In two of these cases a recent thrombotic occlusion of cerebral arteries was demonstrated at autopsy and in three of them a new cerebral infarction was evident. In less than half of the autopsied cases old thrombotic occlusions of cerebral vessels were demonstrated. In two cases post mortem examination established that the original lesion had probably been a cerebral hemorrhage. However in none of them was it found likely that this lesion had contributed to the final outcome. In two of the cases of a fatal cerebral hemorrhage autopsy confirmed that the original lesion had been a cerebral infarction. In the last case this could not be decided.

Chapter V presents comments on the design and the performance of the study and the results. The necessity of following the double blind technique in such studies is emphasized. The study might suggest a poor prognosis for males, for hypertensive individuals, and for those totally incapacitated. Although the two series proved comparable with respect to most general medical and neurological features, it cannot be excluded that slight accumulation of unfavourable factors had occurred in one series. This might have been prevented by making adjustments during the randomization procedure. The importance of taking into account all prognostic factors and of presenting a detailed documentation of the groups is emphasized.

Although the customary limits of statistical significance were not reached, the results do not exclude a moderate protective effect from anticoagulants on the recurrence rate of cerebral infarction. The conclusions were influenced by three additional facts. Firstly the possible risk of thrombo-embolic manifestations during the first months after cessation of anticoagulants may be considered a restrictive factor unless life-long therapy is intended. Secondly it was discouraging that so many cerebral infarctions occurred during periods of an adequate anticoagulant therapy. However this is probably more closely related to the variety of pathogenetic mechanisms which may be involved in the development of a cerebral infarction than to inadequacy of anticoagulants. Thirdly the occurrence of 3 fatal cerebral hemorrhages during the anticoagulant therapy shows that despite reasonable precautions few such episodes seem to be an inevitable complication. These fatalities had decisive influence on the conclusions of the present study.

Similar studies will hardly provide advances in this field. The key to progress rests with an increased knowledge of the processes involved in the development of atheromatosis and thrombosis.

Conclusions

Long-term anticoagulant therapy is without influence on the mortality rate of patients with a completed cerebral infarction of a probable thrombotic and/or atheromatous origin.

This treatment does not influence the neurological rehabilitation in the patients.

A moderate preventive effect from anticoagulants on the recurrence rate of cerebral infarction cannot be excluded, but the danger of provoking fatal complications contraindicates their use.

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SUPPLEMENTUM 437

EXPERIENCES IN THE USE OF DIRECT CURRENT COUNTERSHOCK IN THE TREATMENT OF CARDIAC ARRHYTHMIAS

By

M. Reinikainen P. Koskinen P. Pöntinen and L. Siitonen

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Introduction

Disorders in blood circulation caused by diverse arrhythmias may be most striking, especially if they have lasted for some length of time. For instance, in association with atrial fibrillation, where the heart action is rapid and irregular these disorders are, from the hemodynamic aspect, a most unfavorable condition. Furthermore there is great risk of embolism (6)

Therefore the removal of these disorders at the earliest possible stage is of the greatest importance. However attempts to restore sinus rhythm by means of drugs is not always successful and may even be hazardous, and it is a measure causing severe side effects. As an example may be mentioned that in cases where quinidine has been used in large doses for the reversion of atrial fibrillation, 33 % of the cases have shown toxic reactions (39) Thomson suggested that the probable cause of sudden death is the toxic effect of quinidine upon the central nervous system (48)

It was quite natural that enormous interest was aroused when Lown in 1962 reported new method "for terminating number of diverse ectopic abnormalities of heart beat (20) His first series consisted of 19 patients suffering from arrhythmias of atrial or ventricular origin. In 1956 Zoll and his co-workers reported having used alternating current shock in the treatment of ventricular fibrillation (53) This method has been later successfully used for the treatment of ventricular tachycardia (1, 54) Lown and his co-workers carried out experiments on the effect of alternating and direct current shock on dogs and found that alternating current caused ventricular fibrillation ten times more often than direct current (21) This has been established also clinically in the treatment of arrhythmias, which demonstrates the superiority of direct current to alternating current shock (35)

Up to the present a large number of patients have been successfully treated by this method, which proves its importance as a therapeutic procedure. A number of reports have been published on this subject during the past three years. Lown's first more extensive series consisted of 50 patients with atrial fibrillation, the majority f whom had valvular lesions (22) Sinus rhythm was restored in 90 % of the cases. Already at that time Lown pointed out that the selection of patients for this treatment should follow the principles earlier used

Object of Investigation

The purpose of the present work is to deal especially with the following aspects.

1. To study whether complications occur in association with the conversion of chronic arrhythmias, such as embolism, elevated transaminase levels, ST T changes, and diverse arrhythmias, and what circumstances possibly may be significant in the development of these arrhythmias. Further to examine the maintenance of the result obtained with direct current shock and the significance of maintenance therapy with quinidine and of the dosage of this drug in the persistence of sinus rhythm.

2. To gain experience in the use of direct current shock in refractory paroxysmal tachycardia, including ventricular tachycardia associated with myocardial infarction.

3. To find a suitable and safe anesthetic method for cardioversion.

in determining the use of quinidine for this purpose. Attempts at cardioversion are not justified if the patient does not tolerate quinidine or if sinus rhythm previously restored by drugs cannot be maintained with an adequate dose of quinidine. Lown stated further that car dioversion should not be performed before surgery on patients with an un corrected mitral valvular lesion whose atrial fibrillation has lasted for long er than a year Lown and his co-workers observed further that although sinus rhythm is easily restored by cardioversion, it does not prevent subsequent recurrence of atrial fibrillation. For this reason, maintenance therapy with quinidine is a necessary prophylactic measure (22) Later Lown has in more detail outlined the criteria for the selection of patients with atrial fibrillation for cardioversion. He stated that it is indicated after recovery from successful mitral operation, irrespective of the duration of the fibrillation, and is also justified in patients with nonvalvular heart disease with the exception of elderly asymptomatic patients with a well-controlled heart rate. Also it is indicated in patients whose heart rate cannot be effectively slowed with drugs and in those whose heart failure has been refractory to all measures (23)

In all subsequent reports from various other centers the number of successful reversions has been high, varying between 70 and 93 % in cases of chronic atrial fibrillation (15 18 23 33 34 50) Unfortunately the reversion to sinus rhythm has often been transient only Diverse arrhythmias during a few min utes after conversion have been noted, some of which have led to recurrence of the atrial fibrillation and which account for the transient character of reversion (18)

Chronic Arrhythmias

This group consisted of 63 patients, four of whom had trial flutter and the others atrial fibrillation. There were 34 males and 29 females. The age of the patients varied between 32 and 78 years, the mean age being 58 years. The duration of the arrhythmias varied greatly and it was often difficult to precise it exactly The estimated du ration of the trial fibrillation is given in table I

T ble I Duration f atrial fibrillation

1 month — 11 months	5 cases
1 year — 2 years	18
3 years — 5	13
6 — 10	11
Over 10 years	7
	54 cases

The mean duration of atrial fibrillation was about 5 years in patients converted to sinus hythm. The shortest duration was 1 month and the longest 20 years. In five instances of atrial fibrillation the patient was unable to give any in formation on this point. In atrial flutter the duration generally was shorter Two patients had had it for some months, one patient for 3 years and three patients for 6 years. The mean duration of atrial flutter was 2 years and half.

Twenty four patients (39 %) had atherosclerotic heart disease, which was the most common form of heart disease in this series (table 2) Diverse valvular lesions were responsible for the ar rhythmia in only 17 patients (27 %)

T ble II Underlying heart diseases and successful and failed reversions

Diagnosis	Number of patients	Success	Failure	Number of discharges			
					2	3	
Atherosclerotic heart disease	24	18	6	10	8	2	4
Hypertonic heart disease	4	3	1	3			1
Rheumatic heart diseases							
Mitral stenosis	6	6	—	4	1	1	
Mitral stenosis (post valvulotomy)	4	3	1	2	1	1	
Mitral stenosis and insufficiency	4	3	1	2	1	1	1
Mitral insufficiency	1	1	—	1			
Rheumatic aortic stenosis	2	2	—		1		
Treated myocarditis	1	1	—		1		
Septal trial defect (operated)	1	1	—	1			
Cor pulmonale	2	2	—	1	1		
Treated thyrotoxicosis	9	5	4	4	2		3
Lone (fibrillation or flutter)	5	5	—	5			
Total	63	50	13	33	16	8	9

Material and Methods

The series consisted of two groups of patients on whom restoration of sinus rhythm was attempted by direct current electrical shock. The first group comprised 63 patients with chronic atrial fibrillation or flutter and the second group 19 patients hospitalized for paroxysmal tachycardia. Thus the patients totaled 82 The conversion was usually begun with an energy setting of 100 watt seconds. If sinus rhythm was not produced the DC shock was repeated immediately by increasing the energy discharge successively to 200 300 and sometimes 400 w.s. More than four attempts were never made All the shocks were given with the electrodes in the anterolateral position.

The following clinical and treatment data of the patients in this series were noted down age sex, underlying disease, duration of arrhythmia, attempted conservative conversion with quinidine, premedication with quinidine, anticoagulant therapy use of digitalis, heart volume, intensity and number of DC shocks, the immediate result, transient arrhythmias, maintenance dose of quinidine and the quinidine concentration of serum at follow up examinations.

One patient died 10 hours after DC shock was carried out. She was a woman aged 58 with a history of atrial fibrillation of 12 years' duration. She was hospitalized for cerebral embolism and left-sided hemiplegia, and recovered unexpectedly well from the hemiplegia. An attempt was made to regularize the heart rate with quinidine but without success. DC shock was tried 17 days after onset of the cerebral embolism. Sinus rhythm was restored with one discharge. Anticoagulant treatment was not instituted. The patient died, however 10 hours after cardioversion. The postmortem examination did not reveal a fresh embolus. In the right cerebral hemisphere there were only rests of the previous embolus. It may be worth noting that 17 days prior to DC shock the patient was also given digitoxin and the electrocardiogram showed marked S-T depression and ectopic ventricular beats. Subsequent to DC shock the ectopic ventricular beats increased, being a sign of overdigitalization. It is probable that on this basis the patient developed acute ventricular fibrillation, which was the cause of death since a fresh embolus was not found in the postmortem examination.

Embolism

One patient developed pulmonary embolism 6 hours after DC shock. This patient had a very severe congestive heart failure and rapid fibrillation that caused him severe sensations of palpitation. An attempt to restore sinus rhythm with quinidine was ineffective. A discharge of 200 w.s. brought about sinus rhythm. Anticoagulants were not administered. In one patient the left side of the face and the left vocal chord were paralyzed 3 days after DC shock. She was hospitalized because of pain in the chest. When admitted she had rapid fibrillation and felt considerable discomfort for this reason. DC shock was given immediately and sinus rhythm was instantly restored with one discharge. However the patient died suddenly 5 weeks later and the postmortem examination revealed a fresh myocardial infarction as well as signs of old embolus in the vertebral artery

Elevated Transaminase Level

A serum transaminase determination (SGOT) was made in 44 cases on the DC shock day and on each of the two following days. There were elevated levels in 11 out of 44 patients (25 %) In two of these cases two attempts had been made to restore sinus rhythm. We had been compelled to give three to four discharges to 9 patients, two discharges to one and one discharge to one patient only The patient to whom two shocks were given had at a previous attempt received only one shock, which was not followed by elevation of the SGOT level, but at the second attempt with two shocks the SGOT value increased.

S-T Changes

Six patients showed changes in the electrocardiogram (9.5 %) Four of these also had elevated serum transa

As mentioned above four of the patients in this group had chronic atrial flutter One of these four had atherosclerotic heart disease one a treated thyrotoxicosis and two no underlying heart disease The heart volume was normal in only 16 patients In the other patients it was larger being very large (800—1200 ml/m) in ten

Anticoagulant therapy lasting 10—20 days was given to 46 patients before cardioversion and was not given to 17 patients. Conversion with quinidine was attempted on 45 patients prior to DC shock but failed In four of these cases it had to be discontinued because of nausea and vomiting Only 18 patients were treated with DC shock without a preceding attempt at conversion with quinidine

All except 12 patients were premedicated with quinidine before conversion with DC shock. We have used for this purpose Kinidin Duretter Tablets (Hässle) in a dose of 0.8—1.6 g daily during a few days. Digitalis was given before conversion to all but 14 patients. In 11 instances it was discontinued 2—4 days prior to DC shock. It was administered to 28 patients because of signs of congestive heart failure, and to the other patients to control the ventricular rate. With two exceptions the patients discharged from the hospital in sinus rhythm received a maintenance dosage of quinidine for home use

RESULTS

Fifty patients out of 63 (79 %) were converted to sinus rhythm. As successful conversions were regarded only the cases that still on the subsequent day maintained their sinus rhythm (50) The results of the treatment listed according to the various heart diseases are presented in table II. Six of the successful cases reverted to the original arrhythmia a few days after conversion when the patients still were hospitalized Two of these patients had mitral stenosis two had atherosclerotic heart disease, one had hypertonic heart disease, and one patient was a "lone fibrillator" Of these 6 patients, three did not tolerate the maintenance dose of quinidine. Two patients had severe congestive heart failure at the time the DC shock was given. The sixth patient, the "lone fibrillator" reverted to his original arrhythmia in spite of quinidine premedication and continued administration after the shock. Subsequent attempts were not made Accordingly 44 patients (70 %) were discharged in sinus rhytm.

Table III Arrhythmias following DC shock

	Arrhythmias after DC shock prior to sinus rhythm			Arrhythmias after conversion to sinus rhythm		
	Digitalis therapy			Digitalis therapy		
	Up to DC shock	Discontinued prior to DC	None	U to DC shock	Discontinued prior to DC	None
Atrial ectopic beats				5	1	
Ventricular ectopic beats				8	3	4
Nodal ectopic beats					3	
Atrial and ventricular ectopic beats				2		
Atrio-ventricular dissociation	6	2				
Nodal rhythm	2*)					
Nodal tachycardia	2					
Atrial flutter	1					
Wandering pacemaker	1					
Total	12	2	0	15	7	4

) One of the patients with nodal rhythm had soon thereafter wandering pacemaker between S-A and A-V nodes.

Digitalis was administered to 29 of these 34 patients. The indication in 18 cases was congestive heart failure and in the other cases rapid rate.Digitali zation was discontinued 2—3 days prior to DC shock in the case of 11 patients. No special selection was made of the patients from whom the drug was withdrawn, which was done for the purpose of comparison with the patients who received digitalis up to the time f DC shock. T ble III shows all the ar hythmias and is divided into two sections. On the left are listed the arrhythmias that occurred immediately after cardioversion prior to the restoration of sinus rhythm, and on the right are those that occurred immediately after restoration of sinus rhythm. Both sections are divided into three columns according to whether digitalization was continued up to the time of DC shock or was discontinued a few days previously The third column consists of the patients given no digitalis.

Immediately after the DC shock and prior to sinus rhythm 6 patients out of the 17 who had digitalis until the DC

minase levels. In five patients the changes were depression of S T in the precordial leads in one patient they were extremely striking This patient, to whom two DC discharges were given showed a deep S–T depression in $V_{3,4}$ immediately after conversion, and on the following day there was a negative T_{3-4} The changes had almost disappeared on the next day The SGOT value was elevated to 64 The sixth patient showed intensive elevation of S-T segment in lead I which disappeared within 5—6 hours (fig 1) All but one patient had been given more than one discharge.

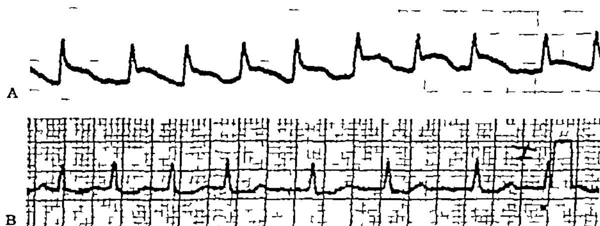


Fig 1 A. Marked ST-segment elevation in a 62-year-old man immediately after DC shock (lead I) B No ST-elevation 6 hours later

Arrhythmias Following DC Shock

Thirty four patients developed diverse arrhythmias immediately after attempt at conversion (54 %) We have examined the question whether digitalis had any responsibility in the occurrence of these arrhythmias (table III) We also studied the effect of various forms of anesthesia in this connection (tables VIII, IX, X) Prior to conversion all but 5 of these patients were given quinidine for a few days. Six patients in table III had several arrhythmias that are detailed in the table.

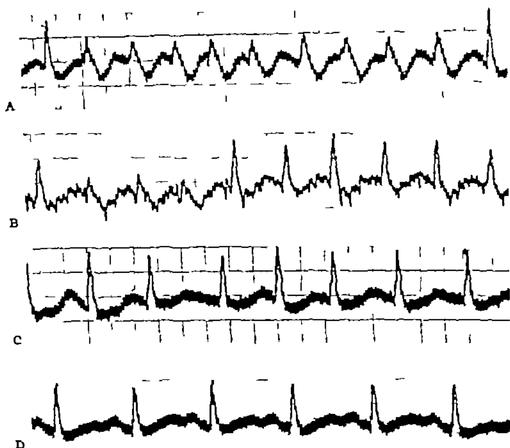


Fig. 2. A man aged 60 with rapid fibrillation and bursts of ventricular tachycardia. A. Prior to attempt at conversion. B. Failure following DC shock (200 w.s.) C. Slowing of fibrillation after injection of propranolol. D. Sinus rhythm after repeated DC shock (lead II)

shock was given showed A—V dissociation (about 36 %) Two of these developed ectopic ventricular beats after reversion to sinus rhythm and one patient *had nodal ectopic beats*. One patient developed a transient rapid atrial flutter that immediately reverted to sinus rhythm spontaneously A—V dissociation occurred in only 2 of the 11 patients whose digitalization was discontinued 2—3 days prior to conversion. After reversion to sinus rhythm one of these had ectopic ventricular beats and sino—atrial block of 2 1 Of the patients that were given digitalis until the DC shock two had nodal rhythm and two nodal tachycardia One of those that at first had nodal rhythm developed ectopic *ventricular beats immediately after reversion* to sinus rhythm In one of the patients with nodal tachycardia it occurred *momentarily immediately after* DC shock after which a wandering pacemaker between the A—V and S—A nodes and occasional atrial ectopic beats were observed.

After restored sinus rhythm there were diverse ectopic beats in 15 out of *the 18 patients (83 %) who were given* digitalis up to cardioversion. They developed in 7 out of 11 patients (64 %) whose *digitalization* was discontinued 2—3 days prior to DC shock.

Out of the 5 patients who had had no digitalis, 4 had ectopic ventricular beats. Each of these had received 3 shocks for restoration of sinus rhythm.

First degree A—V block after DC shock was present in 9 patients, in 7 of whom it disappeared fairly quickly

As a separate observation may be mentioned that already prior to conversion one patient had in addition to fibrillation occasional bursts of ventricular tachycardia (rate 220 per minute) *They persisted* after the DC shock and additionally there were ectopic ventricular beats. The fibrillation persisted. *The patient was given propra*nolol, in response to which fibrillation slowed down and the ectopic beats disappeared. *A further shock brought* about sinus rhythm (fig 2)

As mentioned above, failure at attempted cardioversion occurred in 13 cases out of the 63

Table IV shows the most important data of these patients. Six patients had atherosclerotic heart disease four had a treated thyrotoxicosis, two had mitral stenosis, one of whom had undergone valvulotomy and one had hypertensive heart disease. The mean ag of the pa tients was 58 years, which is approxi mately the same as that of the whole series. Age seems to be of no impor tance, which fact has also been established elsewhere (30) The average duration of fibrillation was 6.2 years (3 patients were not able to state precisely the duration of their ar rhythmia) which is somewhat higher than that of the whole series (5 years) Other workers have made the same observation (29 34 50)

Sinus rhythm was restored in 5 patients after DC shock but only for some hours. Therefore they are not regarded as successful cases f cardioversion (cases 1 2, 3, 6 9) DC shock was given to one of these patients (case 1) immediately after laparotomy as the heart rate and dyspnea increased greatly due to the operation. Evidently for this reason the sinus rhythm was not maintained, her congestive heart failure being quite severe. In addition the patient suffered from severe aci dosis. Patients 2 and 6 had after DC shock very numerous atrial ectopic beats, which evidently is contributed to th recurrence of fibrillation. In case 9 there was a very large heart and a large left atrium. Th cardioversion was attempted 5 months after valvulotomy when the heart was not yet decreased notably in size, and the possibility that sinus rhythm would not maintain was not considered unlikely Of the patients in whom no sinus rhythm was brought about by cardioversion, three had severe heart disease and a large heart volume (cases 5 8, 13) Patient 11 was hospitalized for staphylococcal sepsis and had already previously suffered for a long time from atrial fibrillation. The heart rate increased during fever and the patient was given one DC shock without anesthesia. The patient refused a subsequent shock. In the other cases no essential reason for failure of cardioversion could be shown.

MAINTENANCE OF SINUS RHYTHM

Of the 50 patients whose reversion was regarded as successful (in other words, whose sinus rhythm persisted on the morning following the cardioversion) 37 has remained in sinus rhythm in f llow-up examinations (74 %) The patients followed up for the longest period of time have so far maintained sinus rhythm for 12 months and the most recent cases for one week (table V)

FAILURES

Table IV Failures in attempts to restore sinus rhythm

Case	Sex	Diagnosis additional to atrial fibrillation	Age	Duration of fibrillation	Premedication with quinidine	Anticoagulant therapy	Digitalis up to DC shock	Digitalis stopped prior to DC shock	Heart volume
3	F	Atherosclerotic heart disease Congestive heart failure Ca sigmae	67	7—8 months	—	—	—	—	normal
16	F	Treated thyrotoxicosis	61	3 years	+	—	—	—	normal
20	M	Treated thyrotoxicosis	51	15 years	+	—	+	+	720ml/m^2
21	F	Treated thyrotoxicosis	55	3 years	+	+	—	—	590ml/m^2
24	M	Mitral stenosis and insufficiency Congestive heart failure	34	8 years	+	+	+	+	1750ml/m^2
26	F	Atherosclerotic heart disease Congestive heart failure	55	?	+	+	—	—	630ml/m^2
32	F	Treated thyrotoxicosis	47	1—2 years	+	+	+	+	530ml/m^2
34	M	Atherosclerotic heart disease	68	5 years	+	+	—	—	700ml/m
38	M	Mitral stenosis, post valvulotomy	39	5 years	+	—	+	+	1200ml/m^2
41	M	Hypertensive heart disease Congestive heart failure	56	?	+	+	+	+	normal
42	M	Atherosclerotic heart disease Staphylococcal sepsis	63		—	+	+	+	550ml/m
56	M	Atherosclerotic heart disease Congestive heart failure	62	5 years	+	+	+	+	600ml/m
57	M	Atherosclerotic heart disease	64	15 years	+	+	+	+	1000ml/m

DISCUSSION

The proportion of successful reversions, 79 % was approximately the same as the percentage stated in most other series (15 23, 26, 30 33 34) In the series of Varnauskas et al. cardioversion was successful in 71 % (50)

The mean duration of fibrillation in our series (about 5 years) was higher than that in other observations published so far (23 34) Many of these patients had a very severe case of heart disease. For instance, 10 patients had a heart volume of 800—1200 ml/m This series also includes patients whose fibrillation had lasted for a considerable length of time (longest duration 20 years) Thus it is seen that there was no special selection of patients for correction of arrhythmia by DC shock, in order that additional experience may be gained in the possibility to treat various types of patients with chronic arrhythmia.

It is significant that a relatively large number of failures occurred in patients whose arrhythmia was caused by thyrotoxicosis. Furthermore, many of these patients required relatively many discharges (table II) The attempts at reversion were carried out t a time when the patients had been euthyreotic for some time. The opposite observation was made by Pantridge (34) Thirty-three patients required only one DC shock. All patients who were "lone fibrillators" belonged to this group. On the other hand, Mc Donald et al. observed in their series that in patients with lone artial fibrillation there was an unexplained high relapse rate (26) In the group of 17 patients with diverse mitral valvular disease only 4 of whom had undergone operation for this reason (table II) sinus rhythm was not restored by DC shock in 2 cases only In 2 patients with mitral stenosis the fibrillation recurred 4 days later Both had a large left atrium and they were unoperated cases. Relapse into the original arrhythmia in such cases is a common observation everywhere (15 23, 29)

Premedication with quinidine prior to DC shock is considered important, as it is held to inhibit a relapse into fibrillation (9 18, 22 30, 45) Our observations also suggest this tendency for sinus activity was not restored by cardioversion in 2 of the 12 patients not premedicated with quinidine and four other patients reverted to their original arrhythmia within 2—3 days.

The need for anticoagulant therapy prior to DC shock has been a subject of considerable discussion. Some workers have regarded it as unnecessary (15 34) while others again have given it regularly (9 18, 28) In many quarters the opinion prevails that it is indicated especially in the presence of a risk of embolism (23, 33) However Lown stated that the question of anticoagulant drugs is at present unresolved (23) In our series this therapy was given to 46 patients (73 %)

Table V Maintenance of sinus rhythm restored by cardioversion in 50 patients

Time of observation	Number of patients	Normal sinus rhythm maintained	Reverted to atrial fibrillation
2 — 7 days	10	3	7)
1 month	9	5	4)
1 — 3 months	6	6	
4 — 6	13	11	2
7 — 9	8	8	
10 — 12	4	4	
Total	50	37	13

) 6 patients not given quinidine
) 2 "

Of the 13 relapses to fibrillation 7 occurred during the first 5 days 6 of these had not received quinidine main tenance therapy In 4 patients the *fibrillation* recurred within a month 2 of these had not been given quini dine. In 2 *other patients the sinus* rhythm was maintained for more than 3 months. Atrial fibrillation recurred in 5 patients only (10 %) in spite of maintenance therapy with quinidine One of these patients had mitral stenosis and insufficiency and one had mitral stenosis (in both the heart and left atrium were greatly enlarged) One patient had a treated rheumatic the quinidine concentration in the serum varied from 1 to 4 mg/L (7) Quini dine conversion had first been tried on 41 patients but without success. Of these patients 33 (80 %) were converted to sinus rhythm by means of the DC *shock and in 26 patients (63 %)* it persisted during the whole follow-up period. This result corresponds with that of other workers (30) In 18 out of 22 patients (82 %) whose atrial fibrillation had lasted for less than 2 years the sinus rhythm was maintained. *Of the 28 patients with atrial fibrilla* tion for over 3 years, 15 (54 %) remained in sinus rhythm (table VI)

Table VI Duration f atrial fibrillation

Duration of trial fibrillation	Number of patients	Reverted to trial fibrillation			Sinus rhythm maintained	
		in week	in mo.	after 3 mos.	Patient living	Patient dead
0 — 2 years	22	1	2		18 (82%)	1
2 yrs. o more	28	6	2	2	15 (54%)	2
Total	50	7	4	2	33	4

heart disease and two had atherosclerotic heart disease. Kinidin Duretter tablets were used for the quinidine maintenance therapy in a *daily dose* of 0 8 g Only 8 patients received a larger *dose* (1.2 g) With these doses The corresponding figures in Morris' series were 83 % and 47 % (30)

Among the patients maintained in *sinus rhythm* for more than 3 *months* 17 had a markedly enlarged heart, the volume being over 600 ml/m in 8 patients.

drug employed, and 47 hours when longer-acting agents have been used. Electrolyte deficits, especially potassium depletion, must be corrected before the elective reversion. (23)

It has been observed earlier that ectopic beats occur if large watt second values are required for reversion (23) In our series ectopic ventricular beats occurred in 5 patients who were not digitalized prior to DC shock, but all f these patients had required 3 discharges for reversion to sinus rhythm. This corresponds with the observation above.

First degree A—V block occurred in 9 patients but disappeared fairly quickly in 7 Lown states that this is frequent in patients with long standing fibrillation (23) This statement is supported also by our observations, since the mean duration of f brillation in these patients was 7 years.

As a separate observation we should like to point out that three patients developed, not immediately but two or three days after DC shock, multi focal ectopic beats and bigeminy later after reversion to sinus rhythm. One of these patients had in addition a total A—V block. They had all received moderat doses of digitalis for congestive heart failure up to the time of DC shock. The electrocardiogram revealed nothing special t the time with the exception of fibrillation. Only after performance of the DC shock were there signs of overdigitalization. In addition to the above arrhythmias there was an onset of omiting. Hemodynamic factors evidently had an in direct influence on the digitalis tol erance. The symptoms of congestive heart failure disappeared soon after cardioversion. When digitalis was stopped, the arrhythmias quickly van ished.

In view of the above, special atten tion should be paid to the dosage of digitalis before and after cardiover sion if the patient has an actual need for it, because unexpected signs of overdigitalization may become evident after DC shock.

On this base ventricular fibrillation evidently occurred in the patient that died 10 hours after DC shock (page 11)

In follow-up examinations 37 pa tients were found to have maintained sinus rhythm out of the 50 patients that were dismissed after a successful reversion. For instance, 25 patients have been in control for 4 to 12 months and 23 out of them have maintained sinus rhythm. This may be considered a satisfactory result. An adequate dosage of quinidine is of definite importance, as has been em phasized also lsewhere (2, 19 23, 29) It is well to employ preparations of sustained release (8) in order to get an even 24-hour action with two daily doses, the minimum concentration being 80 ᶜ of the maximum con centration (42) As mentioned above the daily dose of quinidine used by us has been 0.8—1.2 g. and with this dosage the serum quinidine level has varied from 1 to 4 mg/L (7) A number of workers have employed a daily dose of 0.9—2 g (3, 9 23, 29) Some other workers recommended earlier

There are in the literature some statements of the occurrence of embolism subsequent to DC shock (4 23 30 33) On the other hand, there are extensive series, where this condition has not occurred (16 18 25 34) In neither of our cases (1 pulmonary and 1 vertebral artery embolism) had anti coagulant therapy been given as the cardioversion was carried out soon after admission to the hospital.

Elevated transaminase levels subsequent to DC shock are in our series more numerous than elsewhere (15 26 33) with the exception of the series of Cullhed et al., in which they were elevated in 7 out of 30 patients (9) They reported that in cases that need several shocks there may occur changes in the dorsal muscu lature or in the heart muscle, which would explain the elevated transami nase level. We had to give 3 or 4 discharges to 9 patients two discharges to one and only one discharge to one patient The patient who received 2 discharges had in an earlier attempt at cardioversion received only 1 discharge and there had been no rise in the transaminases, but at the second at tempt carried out with 2 discharges the SGOT values were clearly elevated. Our observations suggest that the use of several successive discharges may account for the elevation of trans aminase levels in serum.

Lown et al had produced electro cardiographic changes simulating myocardial infarction in experimental ani mals by alternating current shock. They were also brought about by DC shocks but to a much lesser extent (21) In clinical series, however there is very little information of ST T changes except for an occasional statement in some series of patients (26 32) Sussman et al. have discussed the subject in more detail in describing a case with marked but rapidly passing S–T changes after DC shock. They pointed out that the changes are with out significance because of their short duration (47) In our series there were a relatively large number of ST T changes (6 cases) all of which were transient. From our observations it seems that they are not of impor tance It may be worth mentioning that with one exception these patients had undergone more than one DC shock. On the other hand, Kong and Proudfit reported a patient given at least 150 shocks in whom nevertheless no cardiac muscle injuries were observed (16)

As was mentioned above, diverse arrhythmias were observed in 34 pa tients in association with cardioversion. It is evident that digitalis has some role in the occurrence of these various arrhythmias, for it depresses the S—A node as also does quinidine (14, 18) (table III) The potassium level was determined in 10 of these patients prior to DC shock and it was reduced in 5 which tends to increase ectopic beats. Our observations in this respect correspond with those of Lown. He points out that postreversion arrhythmias can be diminished if dig italis glycosides are stopped for at least 24 hours when digoxin is the

drug employed, and 47 hours when longer-acting agents have been used. Electrolyte deficits, especially potassium depletion, must be corrected before the elective reversion." (23)

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As a separate observation we should like to point out that three patients developed, not immediately but two or three days after DC shock, multi focal ectopic beats and bigeminy later after reversion to sinus rhythm. One of these patients had in addition total A—V block. They had all received moderate doses of digitalis for congestive heart failure up to the time of DC shock The electrocardiogram revealed nothing special at the time with the exception of fibrillation Only after performance of the DC shock were there signs of overdigitalization. In addition to the above arrhythmias there was an onset of vomiting. Hemodynamic factors evidently had an in direct influence on the digitalis tol erance. The symptoms of congestive heart failure disappeared soon after cardioversion. When digitalis was stopped, the arrhythmias quickly vanished.

In view of the above, special atten tion should be paid to the dosage of digitalis before and after cardiover sion if the patient has an actual need for it, because unexpected signs of overdigitalization may become evident after DC shock.

On this base ventricular fibrillation evidently occurred in the patient that died 10 hours after DC shock (page 11)

In follow-up examinations 37 pa tients were found to have maintained sinus rhythm out of the 50 patients that were dismissed after a successful reversion. For instance, 25 patients have been in control for 4 to 12 months and 23 out of them have maintained sinus rhythm. This may be considered a satisfactory result. An adequate dosage of quinidine is of definite importance as has been emphasized also elsewhere (2, 19 23 29) It is well to employ preparations of sustained release (8) in order to get an even 24 hour action with two daily doses, the minimum concentration being 60 % of the maximum concentration (42) As mentioned above the daily dose of quinidine used by us has been 0.8—1.2 g. and with this dosage the serum quinidine level has varied from 1 to 4 mg/L (7) A num ber of workers have employed a daily dose of 0.9—2 g (2, 9 23, 29) Some other workers recommended earlier

considerably larger doses of quinidine for the maintenance of sinus rhythm brought about by cardioversion (at least 1 6 g quinidine sulfate divided into 4 doses (44) or Kinidin Duretter 1 0—3 0 divided into 2 doses (6 8)

Checking of the serum quinidine level is particularly significant as it enables us to establish in time at follow up examinations whether the patient is neglecting to take the drug and to warn him of the consequences. In routine hospital work the individual determination of the quinidine dosage on the basis of the serum quinidine level is too laborious in relation to the benefit gained. In the majority of patients (90 %) sinus rhythm is main tained with a constant moderate dose. An important circumstance possibly influencing the maintenance of sinus rhythm is the duration of the atrial fibrillation. Thus sinus rhythm was maintained in 82 % of the patients in whom atrial fibrillation had lasted for less than 2 years, whereas among those with a history of over 3 years duration the percentage was only 54 %

Heart volume unless greatly en larged had no special significance for the maintenance of sinus rhythm. We should like to state, however that there were three failures of attempted cardioversion by DC shock among the 10 patients whose heart was extremely large (volume 800—1200 ml/m^2) and that three of the latter reverted to the original arrhythmia within 2—3 days after initially successful DC shock.

Paroxysmal Tachycardias

Paroxysmal tachycardias are generally considered to include the following: supraventricular tachycardia, paroxysmal atrial fibrillation, paroxysmal atrial flutter ventricular tachycardia, and ventricular fibrillation. Our series included 4 cases of supraventricular tachycardia, 3 of atrial fibrillation, 4 of atrial flutter 5 of ventricular tachycardia, and 3 of ventricular fibrillation. Most of these patients had a relatively rapid heart rate and some in addition, were critically ill. In our opinion it was important in our cases to get the paroxysmal tachycardia to cease as quickly as possible. For this reason, and since some cases furthermore had proved refractory to antiarrhythmic drugs, we decided to attempt conversion of the rhythm by means of external DC shock. The clinical information and the results of the electrical conversion are summarized in table VII.

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'able VII *Summary of clinical information and results of electrical conversion in 20 pisodes of paroxysmal t chycardia.*

Case N o.	Age and sex	Nature of tachycardia	Underlying disease	Duration of tachycardia	Ventricular rat	Medication prior t conversion	DC ho k		Follow-up
							strength	Result	
64	59 F	Tachycardia supraventricularis	No cardiac disease	4 hours	200	Digitalis, metharaminol	200 w.s.	Sinus rhythm at 120/min.	4 months, normal rhythm
65	57 M	Tachycardia supraventricularis	Old myocardial infarct	26 hours	200	Digitalis	100 w.s. ?00 w.s.	No effect. Immediate slowing of rhythm to 170/min. After 1¼ hours sinus rhythm at 120/min.	4 months, normal rhythm
66	47 F	Tachycardia supraventricularis	Thyrotoxicosis	24 hours	190	Digitalis, quinidine	100 w.s. 200 w.s.	Slowing of rhythm to 165/min. Further slowing of rhythm to 135/min. After 4 hours sinus rhythm at 90/min.	4 months, normal rhythm
67	65 F	Tachycardia supraventricularis	Cerebral vascular accident, hypertension, polycythaemia vera	2 days	170	Digitalis	100 w.s. 200 w.s. 300 w.s.	No effect No effect Slowing of rhythm to 140/min. After 3 hours sinus rhythm at 100/min.	7 months, normal rhythm
68	38 M	Paroxysmal atrial flutter A V block 2 1	No cardiac disease	14 days	140	Digitalis, quinidine	100 w.s.	Sinus rhythm	6 months, normal rhythm
69	57 M	Paroxysmal atrial flutter A V block 2 1	Old myocardial infarct, congestive heart failure	7 days	85	Digitalis, quinidine	200 w.s.	Sinus rhythm	After one week total A-V block
70	49 M	Paroxysmal atrial flutte A V block 2 1	No cardiac disease	3 days	140	Digitalis, quinidine	200 w.s.	Sinus rhythm	1 month normal hythm
71	45 M	Paroxysmal atrial flutter A V block 2 1	Polyserositis, co gestive heart failure	24 hours	150	None	100 w.s.	Sinus rhythm	6 months, normal hythm
72	27 M	Paroxysmal atrial fibrillation	Neurocirculatory asthenia	24 hours	180	N ne	200 w.s.	Sinus rhythm	3 months, normal hythm
73	23 F	Paroxysmal atrial fibrillation	Mitral stenosis, post-valvulotomy	12 hours	180	N n	80 w.s.	Sinus hythm	3 weeks, rmal

74	62 M	Paroxysmal trial fibrillation	No cardiac disease	24 hours	130	None	100 w.s.	Sinus rhythm	Not see afterwards
75	83 F	Tachycardia ventricularis	Post-strumectomy st t	1½ hours	200	None	100 w.s.	Sinus hythm	1 month, normal rhythm
76	70 F	Tachycardia ventricularis	Atherosclerotic heart disease	4 hours	200	None	100 w.s.	Sinus rhythm with umerous xtrasystoles f ventricular origin. After 2 hours atrial fl tter with 4 1 A-V block. After 4 hours return to sinus rhythm	2 months, normal rhythm
77	60 M	Tachycardia ventricularis	Trauma (contusio pedis) N cardia disease	12 days	165	Quinidine, digitalis, ajmalin	200 w.s.	Sinus rhythm	Not seen afterwards
78	61 F	Tachycardia ventricularis	Atherosclerotic heart disease, congestive heart failure	4 hou	190	Procaine amide, digitalis	100 w.s.	Sinus hythm	7 months, normal hythm
79	68 F	Tachycardia ventricularis After 3 days another ventricular tachycardia	Acut myocardial infarct	3 days 3 hours	180 190	Quinidine digitalis procaine amide	100 w.s. 100 w.s.	Sinus rhythm Atrial fibrillation. After 4 days sinus hythm	1 year 3 months, normal rhythm
80	66 M	Fibrillatio ventricularis	Acut myocardial infarct	5 min.	—	None	200 w.s.	Sinus hythm. Aft 5 min. total A V block and very slow idio-ventricular hythm lasting 10 min. and ter minating i asystole	Died 15 min tes fter DC shock. Aut psy Occlusio art.coron cordis
81	52 M	Fibrillatio ventricularis	Acut myocardial infarct	3 min.	—	None	200 w.s. 300 w.s.	N ffect. Si us rhythm at 100/ min. A few ve tric-la extrasystoles	6 weeks, normal hythm
82	70 F	Fibrillatio ventricularis	Atherosclerosis universalis, Ga grena ext inf. (during induction f anaesthesia)	2 min.	—	None	100 w.s.	Immediately after DC-shock trial fib-rillation turning to sinus rhythm	Died f pulmonary mbolism 2 weeks after DC shock and 1 week fter femoral amputation. A topsy: Embolia et infarctus pulmonis

Table VII [illegible] *episodes f paroxysmal ta hycardia.*

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strumectomy for thyrotoxicosis and in the other patient after trauma caused by an iron bar falling on his foot. The heart rate varied between 165 and 250 beats per minute. A markedly enlarged heart (1100 ml/m²) was seen in one patient (case 78). The duration of tachycardia was from 1/2 hour to 12 days. Conservative conversion with antiarrhythmic drugs was attempted in 3 patients prior to DC shock but without success. In case 75 there had been an episode of ventricular tachycardia already during the strumectomy and reversion to sinus rhythm had been rapidly effected with propranolol (Inderal).

The ventricular tachycardia was abolished by DC shock in all 5 patients. One single discharge of 100 or 200 w.s. was sufficient in each case. After reversion one patient (case 76) had numerous ectopic beats of ventricular origin and 2 hours later a 4:1 atrial flutter. Sinus rhythm returned after 4 hours. The patient in case 79 was given a discharge of 100 w.s. during her second episode of ventricular tachycardia. This shock was followed by atrial fibrillation, which later reverted to sinus rhythm. Two patients (cases 75 and 79) showed deep and one patient (case 77) slight inversion of the T waves after tachycardia. The transaminase (SGOT) level increased greatly in one patient (case 79) whose tachycardia was associated with myocardial infarction. All the patients survived and were discharged in a good condition. Four patients have had a follow-up examination and all have maintained sinus rhythm.

Ventricular Fibrillation

Three patients were found to have ventricular fibrillation. The underlying heart disease of 2 patients was myocardial infarction. The third patient was being prepared for leg amputation because of atherosclerotic gangrene when arrhythmia appeared under induction of anesthesia. The arrhythmia lasted from 2 to 5 minutes in these cases. Extrathoracic cardiac massage and artificial respiration were immediately instituted in all cases. The ventricular fibrillation was abolished in all three patients treated. In one case, however there rapidly followed a total A—V block, idioventricular rhythm and asystole. In another patient the DC shock was followed immediately by atrial fibrillation, which turned to sinus rhythm. The patient died of pulmonary embolism one week after the leg amputation and two weeks after the DC shock. The third patient (case 81) whose electrocardiogram is presented in fig. 3 is well and was discharged 5 weeks after the DC shock.

Supraventricular Tachycardia

As stated, there were 4 cases of supraventricular evidently atrial tachycardia. One of these patients had no underlying heart disease one had previously had myocardial infarction thyrotoxicosis was diagnosed in one, and in one patient the tachycardia occurred during a cerebral vascular accident. In one case it had been possible to terminate with digitalis and metharaminol a previous episode of tachycardia on the same day but on its recurrence these drugs were ineffective Attempts to stop the tachycardia in the 3 other patients with vagal maneuvers and digitalis failed Two or more DC discharges were given to all the patients, with the exception of one (case 64) who immediately reverted to sinus rhythm. The immediate result in the other cases was a retardation of the tachycardia, and reversion to sinus rhythm took place only some hours later In cases 66 and 67 the T waves were deeply inverted following the tachycardia. SGOT remained normal. Grave pulmonary edema had developed during tachycardia in two patients (cases 65 and 67) Immediately after slowing of the rhythm following the DC shock there was a marked subjective and objective improvement in the patients condition. All the patients survived and were in sinus rhythm when seen later

Paroxysmal Atrial Fibrillation and Atrial Flutter

Our series included 3 patients with paroxysmal atrial fibrillation and 4 with atrial flutter In 4 of these there was no definite diagnosis of organic heart disease ("lone fibrillation and flutter") One patient had had myocardial infarction and one had polyserositis and congestive heart failure. The onset of atrial fibrillation in one patient occurred 2 days after valvulotomy for mitral stenosis. All of the 4 patients with atrial flutter had a 2 1 A—V block, the ventricular rate varying from 85 to 150 Reversion to sinus rhythm with quinidine was attempted in 3 cases of atrial flutter but failed. In all these cases of paroxysmal atrial fibrillation and atrial flutter cardioversion was successful with one DC discharge varying from 80 to 200 w.s. After the shock 5 patients used quinidine and 3 patients digitalis as a maintenance therapy Six patients in this group have had a follow up examination and 5 of them were maintained in sinus rhythm. One week after cardioversion the sixth patient (case 69) had nausea, vomiting and a total A—V block and was rehospitalized.

Ventricular Tachycardia

Ventricular tachycardia was diagnosed in 5 patients, one of whom had two episodes. The mean age of this group was 63 years (range 55—76 years) There were 4 female and 1 male patient. The arrhythmia was associated with myocardial infarction in one case and atherosclerotic heart disease was present in 2 cases. There was no definite diagnosis of organic heart disease in 2 cases. In one of the latter patients the onset of tachycardia was 2 days after

strumectomy for thyrotoxicosis and in the other patient after trauma caused by an iron bar falling on his foot. The heart rate varied between 163 and 250 beats per minute. A markedly enlarged heart (1100 ml/m²) was seen in one patient (case 78). The duration of tachycardia was from 1/2 hour to 12 days. Conservative conversion with antiarrhythmic drugs was attempted in 3 patients prior to DC shock but without success. In case 75 there had been an episode of ventricular tachycardia already during the strumectomy and reversion to sinus rhythm had been rapidly effected with propranolol (Inderal).

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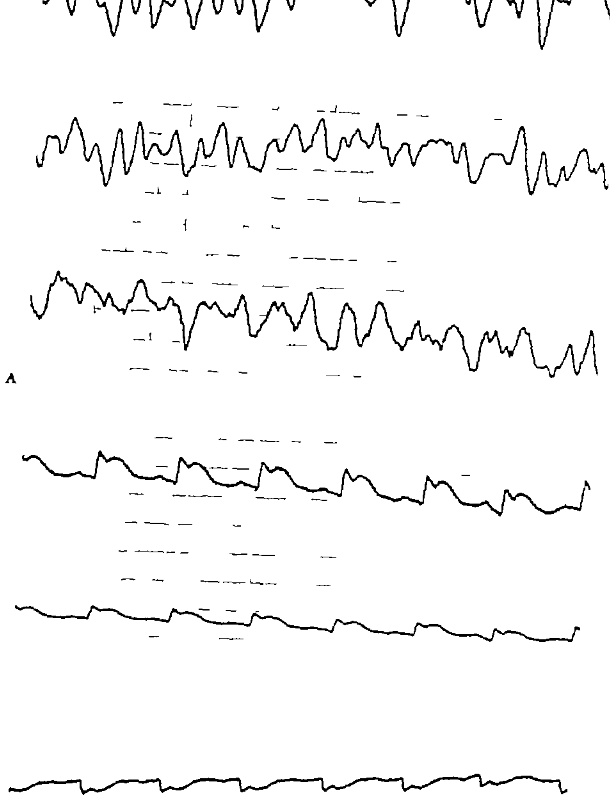


Fig 3 A The patient is 63-year old man (case 31) sufferi g from acut myocardial i farct The ecg reveals a ve tricular fibrillation. B R turn t si hythm after th seco d direct-current cou tershock (leads I II III)

DISCUSSION

Supraventricular tachycardias, the most common form of which is atrial tachycardia, have a sudden onset and frequently cease spontaneously. They are usually harmless, but if the patient has an organic heart disease the tachycardic episode may be accompanied by symptoms of congestive heart failure. In two cases in the present series pulmonary edema of a grave degree developed during tachycardia. The tachycardia of one patient was once successfully terminated with digitalis and metharaminol, but on recurrence these drugs were ineffective. Three other cases were resistant to vagal maneuvers and digitalis. According to Lown (23) cardioversion should be reserved for treating these refractory supraventricular tachycardias. Reversion to sinus rhythm was successful in 7 of his 10 cases. In the 3 failures the ventricular rate was 180 or less. In 1963 Corwin et l. (8) reported the first case of conversion of paroxysmal trial tachycardia with block by electrical counter shock. The arrhythmia had proved refractory to antiarrhythmic drugs but was promptly reverted to sinus rhythm with a single DC discharge. In our series an immediate reversion to sinus hythm occurred in one case only. In three other cases two or more discharges were necessary and the immediate result was learly slowing of the rhythm, but reversion to sinus rhythm did not occur until a few hours later. The transition from supraventricula tachycardia to sinus rhythm spontaneously or f l lowing carotid sinus stimulation can occur likewise in different ways. A posttachycardic pause followed by sinus rhythm is common, but sometimes the transition can be gradual (12).

Atrial flutter has been easiest disorder to terminate with cardioversion (23). Reversion was successful in all of the 7 patients treated by Morris et al. (30) and in the 5 treated by Miller (29). According to the latter a synchronized precordial electrical shock is suitable for the initial treatment of atrial flutter since it is difficult to control this rhythm with drugs.

It is obvious that electrical conversion is warranted in cases of paroxysmal atrial fibrillation and atrial flutter in which there is a rapid ventricular rate as was the case in our series. It is to be recommended all the more for the reason that cardioversion can frequently be brought about by a single discharge.

Ventricular tachycardia frequently occurs in association with an organic heart disease and, especially if of long duration, it has a very harmful effect on the hemodynamics. DeSanctis and Lown give preference to the electrical conversion of ventricula tachycardia, particularly in critically ill patients (10, 23). Lown treated 32 episodes of ventricular tachycardia occuring in 15 patients by cardioversion. Thirty-one (97 %) were reverted to sinus rhythm. In the series of DeSanctis ventricular tachycardia occured 21 times in 12 patients and was successfully converted in 20 episodes. In the one case the shock was followed

by ventricular fibrillation, which ceased after a second shock. Miller's series included 2 successfully converted cases of ventricular tachycardia.

We concur in the opinion of Lown and DeSanctis that electrical cardioversion is the treatment of choice for ventricular tachycardia, especially in patients who are severely ill. It is also indicated for ventricular tachycardia due to myocardial infarction. Electrical countershock quickly stops this form of arrhythmia before deleterious disturbances occur in the hemodynamics. In 3 cases in our series the ventricular tachycardia was stopped by DC shock after lasting for only a few hours. In the light of our limited experience it would seem that conversion of rhythm can be effected more readily in ventricular than in supraventricular tachycardias. In all of the six episodes of ventricular tachycardia arrhythmia was abolished by a single DC countershock. As intervening rhythms were atrial fibrillation and atrial flutter occurring in one instance each.

The mechanism of cardiac arrest is either ventricular standstill or ventricular fibrillation (55) The latter occurs particularly as a complication of myocardial infarction External electrical countershock has been successfully used to stop the arrhythmia. Using alternating current shock, Zoll et al. in 1956 caused ventricular fibrillation to cease 20 times in 11 patients (55) With one exception the patient died, however In Miller's series the reversion was successful in 3 out of 4 patients. Two patients died within one hour from the reversion (29) Sloman et al. presented 3 cases of ventricular fibrillation (43) in 2 of whom acute myocardial infarction was diagnosed. A number of DC shocks were necessary for defibrillation in these cases because of recurrence of the ventricular fibrillation Only after the treatment was supplemented with propranolol (Inderal) a beta receptor blocking agent, was the arrhythmia effectively under control. One of their patients survived and was in good condition 6 weeks after the cardiac arrest. In present series one patient survived and was discharged 5 weeks after the DC shock.

It is obvious that cardiac resuscitation can be successfully carried out by electrical countershock when ventricular fibrillation is the cause of the cardiac arrest. Zoll stated that in the fatal cases the countershock was applied after considerable delay (5 minutes or more) Immediate recognition of ventricular fibrillation depends upon continuous monitoring of cardiac activity It is therefore necessary that the critically ill cardiac patient is continuously under observation in special cardiac units of intensive care.

Anesthesia for Cardioversion

Problems of anesthesia for cardioversion have become topical since this form of treatment has gained general acceptance as a safe and effective method of terminating and inverting previously often fatal chronic arrhythmias. The patients are mostly bad risk cardiac patients. Although the procedure itself lasts only a fraction of a second and the physical trauma which it causes is minimal, the psychic stress is so pronounced that some form of anesthesia is mandatory. The muscle contractions caused by the alternating current (AC) shock formerly in common use had to be abolished by the use of muscle relaxants. The muscle contractions due to direct current (DC) shock are considerably less intensive and the use of muscle relaxants is usually not necessary for the continuous and adequate oxygenation of patients (15 18 52). Some authors, however have used muscle relaxants for better oxygenation before the shock, with or without intubation (41). Cardioversions without any form of anesthesia have shown that the pain it causes is quite tractable or that there is no pain sensation at all. Stock reported 14 cardioversions without anesthesia, during which the patients had only minor unpleasant feelings (46). The muscle contractions and the reactions of patients are naturally comparable to the intensity of the electric shock. A DC shock of 100 w.s. has been proposed as the upper limit of shocks without anesthesia (15). It is wise to remember that although shocks of greater intensity may sometimes cause severe muscular cramps with fatigue and pain in these muscles afterwards the fasciculations after depolarizing muscle relaxants are often followed by muscle pain.

Requirements for anesthesia suitable for cardioversion and possible methods to fulfil them

A suitable anesthesia fulfils the following requirements.

1) is as saf for the patients as possible-

2) does not induce new arrhythmias,

3) involves no risk of explosion,

4) permits adequate oxygenation

5) is short acting but easy to prolong for at least 20 minutes

6) is simple and safe enough to allow the cardioversion to be performed in the ward.

The explosion risk eliminates ether and cyclopropane.

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The explosion risk eliminates ether and cyclopropane.

Halothane although rapid in action and quite pleasant to patients, enhances vagal tone and induces vagal arrhythmias and hypotension. It is also said to induce ventricular arrhythmia, and especially the combination of halothane and adrenaline or other pressor agents often used to terminate tachyarrhythmias has been proved to be potentially dangerous in man (3 17 28 40)

Nitrous oxide-oxygen, when used to induce analgesia and amnesia means such a low oxygen content in the inhaled mixture that a fall in the oxygen tension of arterial blood is inevitable

Neuroleptanalgesia gives good analgesia and amnesia and the patient feels disconnected from the outer world although he is awake when neuroleptanalgesia is used without the addition of nitrous oxide Induction with neuroleptanalgesia is prolonged to 10—15 minutes, and the doses needed for disconnection without the addition of nitrous oxide depress respiration (38) Otherwise neuroleptanalgesia is well suited for geriatric and bad risk patients, and also for patients receiving antihypertensive drugs as its effect on the cardiocirculatory system is minimal (31) Since about fifteen minutes are needed to replace plasma nitrogen with oxygen, intermittent positive pressure breathing with 100 per cent oxygen during the induction of neuroleptanalgesia guarantees a high arterial oxygen tension at the moment of cardioversion.

With intravenous ultrashortacting barbiturates, pentothal or methohexital, anesthesia is induced rapidly but is adequate only for one or two shocks. Additional doses prolong the anesthesia and at the same time enhance the risk of hypotension and laryngeal spasm as well as the cumulation of narcotic effect. Barbiturates do not give analgesia but they diminish muscle contractions. Atropine is recommended for premedication and, especially if methohexital is used opiates also (11) The advantages of intravenous barbiturates are their rapid and short action for a single shock and the easy induction and minimal postoperative sickness. A short acting muscle relaxant must be on hand to combat possible laryngeal spasm (27)

If neuroleptanalgesia and atropine are used for premedication, it is possible to give the first shock of lower intensity (80—100 w.s.) during oxygenation without barbiturates or with an additional dose of neuroleptanalgetic drugs. If the first trial is a failure it is better to continue the procedure under barbiturate anesthesia. A very small dose of pentothal (100—200 mg) or methohexital (30—50 mg) prolongs the anesthesia up to 20 minutes after premedication with neuroleptanalgetic drugs and gives time for as many shocks as are needed for the most insensitive cases.

Effects of simultaneous drug treatment

Hypertensive patients treated with reserpine or guanethidine are very sensitive to barbiturate anesthesia. Small doses of intravenous barbitu

rates sometimes are followed by extensive hypotension which is not corrected by noradrenaline releasing vasopressors. Noradrenaline itself maintains the blood pressure at a reasonable level and, on the contrary, there is sensitiveness to the effects of noradrenaline during reserpine treatment (36). Digitalization increases the possibility of ectopic rhythms and the concomitant hypopotassemia especially is a cause of ectopic beats and other signs of overdigitalization after cardioversion. The origin of these arrhythmias may lie on the vagal effect of the electric shock on the sinoatrial pacemaker and the simultaneous slowing of conduction in the atrioventricular node by digitalis. Hypopotassemia and vagal influence together provide ideal conditions for atrial fibrillation to occur (49). Sympathetic beta-receptor blockers effectively abolish ventricular ectopic beats, but in order to combat vagal tone atropinization is necessary before beta receptor blockers (13, 24, 51). Hypopotassemia, most often induced by diuretics, should be corrected before cardioversion when possible. Beta-receptor blockers may be included in the premedication of patients who have rapid arrhythmias, whose arrhythmia is due to thyrotoxicosis, or who for other reasons are very nervous (37).

PERSONAL EXPERIENCES

A. Anesthetic Technique

The standard premedication has consisted of 0.05—0.10 mg of phentanyl, 5.0—10.0 mg of dehydrobenzperidol, and 0.4—0.6 mg of atropine given intramuscularly half an hour before cardioversion. Ten to 15 minutes of intermittent positive pressure breathing with 100 per cent oxygen from a Bennett apparatus has been the routine before shock. During oxygenation additional doses of phentanyl (0.5—0.20 mg) and dehydrobenzperidol (2.5 —10.0 mg) have been given intravenously when only a single shock has been deemed necessary. If repetition of the procedure has been required the patients have been anesthetized with intravenous barbiturates (pentothal 100—200 mg or methohexital 30—60 mg). In these cases assisted or controlled respiration has been needed until the effect of the barbiturates has waned. If multiple shocks have been anticipated the induction has been with barbiturates. Premedication with neuroleptanalgetic drugs prolonged the anesthesia to outlast the procedure. Predominantly the cardioversions have been carried out in the wards, only two cardioversions having been done in the recovery room, two in the operating theater and two in the casualty department.

B. Results

The results of the first 69 successful cardioversions are presented in the following tables (tables VIII IX, X) the 13 failures are not included.

Anesthetic complications have been limited to temporary respiratory arrests and depressions, which have been easily overcome by intermittent positive pressure breathing with a Bennett venti lator

Table VIII Cardioversions without anesthesia

A Paroxysmal arrhythmias

ythmia	Intensity of D-C shock in w.s.	Result	Intervening rhythms: Atrial ectopic beats	Ventric. ectopic beats	Nodal ectopic beats	Atrial & ventr ect. b.	A V dissoc.	Nodal rhythm	Nodal tachycardia	Atrial flutter	Wandering pacemaker	A V block	S-A block	
ricular fibr	100	NSR)												
	200	NSR										1		death in 5 min.)
	200+300	NSR												
xysm. atr lation	80	NSR												
aventricular rcardia	100+200	NSR												
B Chronic arrhythmias														
1 flutter	100	NSR												
1 fibrill.	100	NSR												
	200	NSR												

sinus rhythm

loversion was performed during resuscitation afte at least ten minutes anoxe- due to cardia arrest.

Table VIII
Cardioversions performed without anesthesia. Seven patients were conscious but had only minor unpleasant sensati ns. One of the cardioversions for ventricular fibrillation was performed in the operating theater th second on in the ward during resuscitation in a desperate case, and the last one in the emergency ut-pati nt clinic. Third degree A V block followed the successful restoration of sinus rhythm in the second case.

T ble IX Cardioversions under neuroleptanalgesia

A Paroxysmal arrhythmias

Number of patients	Arrhythmia	Intensity of D-C shock in w	Result	Intervening rhythms: Atrial ectopic beat	Ventric. ectopic beats	Nodal ectopic beats	Atrial & ventric. ect. b.	A V dissociation	Nodal rhythm	Nodal ta hycardia	Atrial flutter	Wandering pacemaker	A V block	S-A block
2	Ventricular tachycardia	100	NSR											
	B. Chronic arrhythmias													
3	Atrial flutter	100	NSR					1						
5	Atrial fibrill.	100	NSR											
2		200	NSR											
3		100 + 200	NSR		1	1		2						1
1		200 + 200	NSR		1									

Table IX
Sixteen cardioversions were performed under neuroleptanalgesia. Four patients were given two successive shocks

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›le VIII

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DISCUSSION

In the light of previous literature and supported by personal experience it seems certain that cardioversion itself is very rarely a dangerous procedure and that it is always possible to perform it at least once without anesthesia. In life-endangering tachy arrhythmias there are no anesthetic problems and all measures should be directed to better oxygenation of the myocardium. On the contrary it is reasonable to anesthetize patients dur ing cardioversions necessitated by supraventricular tachycardia or atrial fibrillation, as there is a greater possi bility of successive shocks being needed. During anesthesia reflex activity increases and after the shock an enhanced vagal tone together with possible digitalization and hypopotassemia provide good conditions for new arrhythmias and especially for vagal intervening rhythms. As hypoxia and hypercapnia increase the risk of ar rhythmia, adequate oxygenation and ventilation are needed before and after cardioversion. A heavy premedication with neuroleptanalgetic drugs and partial tropinization usually provide good conditions for single shock, and if successive shocks are necessary intravenous barbiturates quickly in duce adequate anesthesia. Afterwards this drug combination guarantees a retrograde amnesia and the patient has no unpleasant memories of the procedure. It is wise to remember that the more dangerous or unpleasant the arrhythmia is, the more satisfied will the patient be after successful cardioversion, and this does not depend on the anesthesia.

The need for anesthesia depends greatly on two things the type of arrhythmia and the general condition of the patient.

1) In a case of *ventricular fibrilla tion* the only measures necessary before cardioversion are artificial venti lation and extrathoracic cardiac massage. No anesthesia is needed and the cardioversion must be done as quickly as possible. Direct current shock is better than alternative current shock on a fibrillating hypoxic myocardium and for this reason there is no necessity to try to oxygenate the myocardium beforehand (21 52)

2) *Ventricular tachycardia* is usually easy to terminate by direct current shock of low intensity If the patient is in a state of acute heart failure the anesthesia lowers the cardiac output and unnecessarily increases the risk. Intermittent positive pressure breathing with 100 per cent oxygen and premedication with neurolept analgetic drugs provide ideal condi tions for successful cardioversion. Car dioversion almost immediately restores normal circulation and the patient is slightly euphoric after the shock and tranquilized by the neurolept drugs.

3) *Supraventricular tachycardias* are more resistant to cardioversion and many attempts are often needed to restore normal hythm. As the patients

Table X Cardioversions under barbiturate anesthesia

Number of patients	Arrhythmia	Intensity of D-C shock in w.s.	Result	a	b	c	d	e	f	g	h	i	j	k)
	A Paroxysmal arrhythmias													
3	Ventricular tachycardia	100	2 NSR 1 Atr fibr											
1	Supraventr tachycardia	100	NSR											
1		200	NSR											
1		100+200	NSR											
2		100+200+300	NSR											
1	Paroxysm. atr fibrillation	100	NSR											
1		300	NSR											
1	Paroxysm. atr flutter	200	NSR											
	B Chronic arrhythmias													
1	Atrial fibr	80	NSR		1									
5		100	NSR	1	2	2				1				1
10		200	NSR	3	4		–	1				1		
4		300	NSR					1						
4		100+200	NSR		2				2					
1		200+200	NSR											
4		200+300	NSR	–	2			1		1				
1		100+200+300	NSR					1						
1		80+100+200	NSR		1									
1		80+200+300	NSR		1									
1		80	NSR					1			1			

) = one pati nt was treated twice
) a = Atrial ectopic beats
b = Ventric. ectopic beats
c = Nodal ectopic beats
d = Atrial & ventric. ect. b.
e = A V dissociation
f = Nodal rhythm
g = Nodal tachycardia
h = Atrial flutter
i = Wandering pacemaker
j = A V block
k = S-A block

Table X
42 patients were treated unde barbiturate anesthesia, two of them twice. Intervening rhythms were most common in this group

Thirteen cases were primary failures. These patients were adequately premedicated and anesthetized, which is evident from a small number of intervening rhythms. The number of trials and the intensity of shocks were selected according to the size and condition of the patients. In 5 cases there was successful conversion to normal sinus rhythm with 200—300 w.s. but after some hours there was a relapse to previous arrhythmia.

Summary

The presented series consisted of 82 patients, of whom 63 patients with chronic atrial fibrillation and flutter belonged to the first group and 19 patients with acute tachycardia to the second group.

The mean age of the patients with chronic arrhythmia was 58 years. There were 34 males and 29 females.

The mean duration of atrial fibrillation in the patients reverted to sinus rhythm was 5 years and the duration of flutter 2½ years. Atherosclerotic heart disease was the underlying cause in 24 patients (39 %). Diverse valvular diseases were responsible for the arrhythmia for 17 patients (27 %)

Forty-six of the 63 patients received anticoagulant treatment prior to DC shock. Attempts at conversion with quinidine were made in 45 patients but failed. Quinidine premedication was given to all except 12 patients prior to DC shock. Digitalis was administered to all but 14 patients, and in 11 cases it *was stopped 2—4 days prior to DC* shock.

Fifty patients (79 %) were reverted to sinus hythm. Forty three patients (70 %) were discharged from hospital in sinus rhythm.

One patient with treated embolism died 10 hours after DC shock. The post mortem examination showed no fresh embolism. Two patients developed embolism after DC shock in one it was pulmonary and in the other in the vertebral artery

Serum transaminase determinations were carried out in 44 patients. The values were elevated in 11 patients (25 %) S—T changes were observed in 6 patients (9.5 %) and 4 of these had an elevated transaminase level in the blood. The changes disappeared quickly All except one had undergone more than one DC shock.

Diverse arrhythmias appeared immediately after conversion in 34 patients (64 %) They were markedly more frequent in patients given digitalis up to the time of DC shock than in those whose digitalization was stopped 2—3 days prior to DC shock. Ectopic ventricular beats were present in 5 of the patients who had had no digitalis. Each of these had undergone 3 shocks for the restoration of sinus rhythm.

First degree A—V block was present in 9 patients, but it disappeared fairly quickly from 7 after DC shock.

According to follow-up examinations, sinus rhythm has maintained up to the present in 37 patients (74 %) Patients

are usually nervous and not in a life-endangering condition there are sufficient reasons for adequate premedication and for intravenous barbiturates *to facilitate the cardioversion for as* many times as are needed to terminate the arrhythmia.

4) *Atrial flutter is readily reversed* to sinus rhythm, and since a single shock of low intensity is commonly all that is needed, premedication with neuroleptanalgetic drugs is well suited for this procedure It permits additional shocks after a small dose of barbiturate.

5) Chronic *atrial fibrillation* is the most common of the arrhythmias treated by cardioversion. The ultimate *result and the number of attempts* needed are often difficult to foresee. Premedication with neuroleptanalgetic drugs, *intermittent positive pressure* breathing with 100 per cent oxygen, and a small dose of barbiturate maintain adequate anesthesia for successive shocks.

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controlled for the longest period of time have remained in sinus rhythm for 12 months, and the most recent cases for one week. *For instance 23 of 25 patients* who have been in control for 4—12 months have remained in sinus rhythm. The quinidine maintenance dosage has been 0 8—1.2 g The serum quinidine level has been in the range 1—4 mg/liter

Our series included 19 patients with paroxysmal tachycardia in 4 of whom it was supraventricular With one exception all these four patients received two or more discharges of DC shock. The immediate consequence of the shock was in all but one cases a slowing of tachycardia and only after a few hours a reversion to sinus rhythm.

Paroxysmal atrial fibrillation and atrial flutter was present in 7 cases. In all these cases sinus rhythm was brought about with a single discharge. Five patients had used quinidine and 3 other patients have had a maintenance dose of digitalis after the shock.

The heart rate in 5 patients with ventricular tachycardia varied from 165 to 250 beats per minute. The arrhythmia was terminated with a single DC shock in all of these patients.

Ventricular fibrillation was diagnosed in 3 patients, 2 of whom had myocardial infarction as the underlying disease. The duration of arrhythmia varied from 2 to 5 minutes. In all cases extrathoracic cardiac massage and artificial ventilation were immediately begun. The ventricular fibrillation was terminated in all cases by DC shock. In one case, however total A—V block and idioventricular rhythm and asystole followed DC shock.

Six paroxysmal and 6 chronic arrhythmias were treated by cardioversion without anesthesia. Two paroxysmal and 19 chronic arrhythmias were treated under neuroleptanalgesia. Intravenous barbiturate anesthesia was used for 11 DC shocks performed for paroxysmal and for 38 performed for chronic arrhythmia. There were no anesthetic complications. It seems to be always possible to perform cardioversion without anesthesia at *least once using* shocks of a intensity below 200 w.s. When anesthesia is required the most suitable form is intravenous barbiturate anesthesia and premedication with neuroleptanalgetic drugs and atropine

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SUPPLEMENTUM 436

A STUDY OF CARRIERS OF STAPHYLOCOCCUS AUREUS

WITH SPECIAL REGARD TO QUANTITATIVE BACTERIAL ESTIMATIONS

BY
CLAUS OLA SOLBERG

ACTA MEDICA SCANDINAVICA

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A Study of
Carriers of Staphylococcus Aureus

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with Special Regard to Quantitative Bacterial Estimations

By

CLAUS OLA SOLBERG

Preface

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I am deeply indebted to Professors Johs. Bøe and Th. M. Vogelsang, and express my heartfelt gratitude for their great interest in the work and their valuable advice and criticism concerning the preparation of the manuscript.

The skilled and conscientious technical assistance of Mrs. G. Fretheim, Miss B. Östervold and Dr. A. Wormnes was of utmost importance to the performance of the experiments.

I owe my gratitude to the staff of doctors, nurses and assistants in The Medical Department B and the staff of The Supply Department, The Gade Institute who were unfailingly kind and helpful. The secretarial assistance given by Miss K. Aa. Erdal is greatly appreciated.

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Claus Ola Solberg

CONTENTS

I Introduction and plan of study

Before aseptic methods were brought into use, the frequency of infections among patients in hospitals was extremely high. Today hospital infections are far less common, but in relation to the great progress in medical research in the last decades they still represent a major problem.

A substantial number of hospital infections are caused by yellow coagulase positive staphylococci — Staphylococcus aureus (here abbreviated to Staph. aureus) (149). These organisms are extremely widespread both inside and outside hospitals. Fifty to seventy per cent of hospital personnel and thirty to fifty per cent of the external population carry these organisms in the vestibule of the nose (87, 117). Staphylococci are also frequently isolated from the throat, faeces, vagina, axillae, perineum and other skin sites (14, 17, 19, 56, 87, 133) as well as from the air, bedclothes, furniture and floors of the wards (56, 87, 99, 123).

Certain staphylococcal strains seem to be more virulent than others and individuals harbouring such strains are assumed to be dangerous carriers. However, it is reasonable to assume that there is also a quantitative aspect in the development of staphylococcal infections.

Some staphylococcal carriers — the so-called "heavy dispersers" — are able to shed far larger numbers of these organisms than other carriers (55, 99). The mode of transmission of staphylococci is well known (57), but it is not clear why carriers differ in their ability to disperse the organisms into the air (149).

The aim of the present study has primarily been to investigate this quantitative aspect, to study the question of why some staphylococcal carriers disperse much larger numbers of organisms into the air than others.

Measures for the prevention of staphylococcal dispersal in hospitals have frequently been investigated (40, 42, 43, 63, 101, 110, 117). Both personnel and patients have been treated with antibiotics and skin disinfectants. The reduction of bacterial air contamination has been attempted by means of ultraviolet irradiation, glycol vapour, improved ventilation, oiling of floors and bedclothes etc.

The majority of investigations have been undertaken on groups of individuals, e.g. patients in one or more hospital wards. The reduction of bacterial air contamination, frequency of staphylococcal nasal carriers and septic lesions have been indicators of the effectiveness of the treatment (42, 43, 63, 110, 117).

In only a few investigations has attention been focused on the individual carrier (98, 132). Little is therefore known about the ability of the various remedies to reduce the dispersal from carriage sites and septic lesions, the only true sources of

hospital staphylococci. In the present study the effect of antibiotic nasal spray and hexachlorophane skin disinfection have been investigated with a view to reducing staphylococcal dispersal from individual carriers.

To study these problems, it has been necessary to develop suitable methods for quantitative estimations of Staph. aureus on different sites of the body and for assessment of the ability of individuals to disperse the organisms into the air.

II Methods

A. Methods of quantitative estimation of Staph. aureus on different skin areas.

Price (103 104) and Lovell (81 82) divided skin bacteria into two groups, "transient and resident" flora.

The first group comprises all bacteria which are conveyed to the skin from the surroundings. These organisms are usually loosely attached to the surface of the skin. They are relatively easily removed by ordinary washing with soap and water scraping the skin with knife or rubbing with moistened swab. They are present in greatest numbers on the uncovered parts of the body and particularly under the nails (1) while far fewer organisms can be isolated from covered skin areas. The group includes both saprophytes and various pathogenic bacteria such as gram-positive cocci and enterobacteria. Transient organisms may become resident this sometimes occurs when the skin is exposed to the prolonged action of infected material.

The resident organisms multiply on the skin. They lie in the outer layer of the epidermis, in the pilonidal follicles and sebaceous glands, but has not been demonstrated in the sweat glands (81) The number of resident organisms seems to be fairly constant for the same individual from time to time (103) They cannot be completely removed by mechanical and chemical means. Generally they comprise three groups of saprophytes micrococci, corynebacteria and Propionobacterium acnes (85).

1 *Previous methods*

The total number of bacteria on a skin area cannot be measured. By the methods vailable only part of the skin flora can be examined.

In order to assess the effect of skin disinfectants, Price (103) developed a standardized form of hand washing The hands were washed with soap and brush in a series of bowls of water and the bacterial count for each bowl was determined. Nearly all the transient bacteria were washed off in two minutes and the total skin flora was reduced by about a half every six minutes. Price (103) maintained that theoretically it would take about two hours of continuous washing to sterilize the skin. His method is probably the most exact for quantitative estimation of the bacterial flora of the hands, but it is very time consuming Other investigators (20 37 106) have used the same method in principle but with fewer washing steps. In still other investigations (83 84) a standardized form of hand washing without scrubbing has been employed.

Skin contamination has also been assessed by pressing the culture medium against the skin and counting the resultant number of colonies (49 58, 79 118) This method lends itself best to the investigation of the bacterial flora of the hands.

Rubbing the skin with moistened swabs subsequently plated on solid media is a method which has often been employed (30, 39 54 55 87) Alginated swabs have been dissolved for example, in Ringer

solution from which counts of viable organisms have been made (9)

Abrahmson and Smorodinzeff (1) rubbed the skin with sterile gauze and Hegler (70) used a hemp or silk thread Colebrook (24) swabbed the fingers with sterile broth making cultivations in poured agar and on the surface of solid media Burtenshaw (16) applied a rectangular glass chamber to the skin, added saline and scraped the skin with a glass slide to make a suspension of organisms from which he made viable counts. In principle the same method has been used later (69 84 122) Several authors (13 83) have counted the number of organisms in surgical gloves after use for a given period

The accuracy of these methods has seldom been investigated Price (103) examined the results of rubbing the skin with sterile swabs and scraping with a sterile scalpel He found that "transient bacteria, whether involuntary contamination or test bacteria could be pretty effectively recovered by these means, although the results from a quantitative standpoint were not dependable In testing the resident flora, however scraping and rubbing (particularly the former) proved unreliable even from a qualitative standpoint In these investigations Price did not, however describe how the quantitative determinations were performed

The results probably vary considerably from method to method the variations for each individual method being smaller

2 *Personal methods*

In the present study two methods of quantitative estimation of Staph. aureus on the skin have been employed In one method, different skin areas were examined by means of moistened cotton wool swabs. The other method was a standardized form of hand washing

a. Equipment.

Swabs The swabs were made of steel wire and cotton wool. The steel wire was 20 cm long The cotton wool which was wound tightly round one end of the wire, had a diameter of 0.5 cm, the length varying from 2 to 10 cm depending on the size of the skin area to be examined

Test tubes 16 cm long test tubes with an internal diameter of 14 mm.

Pipettes Calibrated pipettes, capacity 0 1 1 and 10 ml

Fluids 0.9 per cent saline was used for all dilutions and for moistening the swabs. Ordinary tap-water was used for washing the hands

Washing bowls These were about 15 cm deep and held 4 litres.

Measuring cylinder A 500 ml cylinder was used to measure the washing water

Brushes Ordinary nail brushes were employed

All equipment was sterile

b Description

Method of investigating different skin areas

The skin areas were firmly rubbed six times with swabs moistened in 0 9 per cent saline. Every second time the swabs were rotated about 120 Two swabs were used for the same skin area one after the other

After obtaining the sample the swabs were put into a test tube containing 10 ml of 0 9 per cent saline and treated in the following manner the steel wire was held between the first and second fingers and rotated 20 to 30 times each way as fast as possible until the cotton wool was freed from the wire. The cotton wool was then gathered round the steel wire and squeezed against the wall of the tube to express the fluid. These operations were performed 5 times with each swab The swabs were then transferred to a second test tube with 10 ml

of 0.9 per cent saline and treated in the same way

The contents of the tubes were mixed and several of the following volumes of the original suspension incubated on mannitol salt agar plates 1/20 1/40, 1/80 1/1,000 1/4 000 1/40,000. Each plate was incubated with 0.25 ml suspension. For each sample 1/20 1/40 or 1/1 000 of the original suspension was always incubated together with one or two of the other volumes, depending on the presumed degree of skin contamination.

Clean pipettes were used for each dilution tube. The incubates were distributed over the medium by shaking the plates horizontally. After 10—20 minutes the fluids had dried into the media and the plates were put in the incubator with the lid downwards. After incubation for 48 hours at 37°C the Staph. aureus colonies were counted and the number of organisms in the original suspension calculated. If a plate yielded more than 200—300 Staph. aureus colonies, one which was incubated with a smaller volume of the original suspension was counted.

Sample from the upper lip. The sample was obtained from 4 sq. cm area in the middle of the upper lip just below the nose.

Sample from the fingers. The sample was obtained from under the nails and proximally from an area 3 cm wide and 2 cm long on the palmar surface of the fingers. Four swabs were used for each hand, 2 swabs for the 1st and 2nd fingers and 2 swabs for the other fingers.

Sample from the perineum and extra-perineal area. As far as possible the precise perineal area examined in women was that between the anal orifice and the vaginal introitus and 3—4 cm to each side of the mid-line and in men the area between the anal orifice and the posterior fold of the scrotum and 3—4 cm to each side. The extra-perineal area constituted here a 5 cm broad field outside the perineum on both sides.

Sample from the axillary region. The sample was obtained from a 50 sq. cm area in the central part of each axilla.

Sample from the skin of the abdomen. The sample was obtained from 200 sq. cm of the skin in the umbilical region.

Sample from staphylococcal infected skin lesions. The whole infected area was sampled, but on 3 occasions these areas were so large that samples were only taken from a smaller part.

Sample from the auditory meatus. The swabs were rubbed around the walls of the auditory meatus.

Method of investigating the hands

This method is the same as that described by Lowbury et al. (84) except for minor modifications.

After obtaining the finger samples, the subjects washed their hands in half a litre of water at 40°C. Both hands were thoroughly moistened up to the wrist and then rubbed firmly 3 times palm to palm, 3 times with the right palm over the left dorsum, 3 times with the left palm over the right dorsum and 3 times with the fingers interlacing. The hands were thoroughly rinsed between each of these manoeuvres. The whole procedure was repeated 5 times in the same water. The subjects were supervised throughout the washing, which lasted about four minutes. The following volumes of the water were incubated: 1/500 1/2 000 1/10 000 and occasionally 1/100,000. Each plate was incubated with 0.25 ml suspension.

c. Reproducibility

In patients who were non-carriers of staphylococci (samples obtained from the

solution from which counts of viable organisms have been made (3)

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c. Reproducibil ty

In patients who were non-carriers of staphylococci (samples obtained from the

Table 1 *Per cent of test bacteria recovered from upper lip of 15 individuals*

No of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		46.3	
14 1		29.3	
12.2	12 4	63 7	49.3
11.9		50.5	
12.1		56.6	
4 6		24.0	
4 4		38.7	
4.8	4.5	42 7	40 6
4.2		21 4	
4.5		56 0	
9 4		57.3	
8.3		60.8	
8.6	9 1	18.1	44.3
9 4		56.8	
9.6		28.2	

nose throat upper lip axillae, skin of abdomen and perineum) the described areas of upper lip 1st and 2nd fingers of one hand skin of abdomen axillae and perineum were infected with 0 04 ml and the hands with 1 ml of a suspension of Staph aureus in physiological saline. For each skin area 3 groups of 5 subjects were infected and for each group the numbers of infecting organisms were calculated in five samples of the suspension used

After the skin had dried for 5 minutes, samples were obtained by the methods described The results are given in tables 1—6 Within each group the variations in the numbers of organisms used for infection were small, but the numbers of organisms isolated from the different subjects varied to a much greater extent. However compared with the results of the later investigations (chapters IV V VI and VII) these variations were of minor importance.

In the later examination of staphylococcal carriers the distribution of the counts from the skin, air and nasal samples was skewed, as commonly occurs in such data (51 86 147) The frequency distribution of the counts was found to approximate the log normal form. For statistical analysis the counts were therefore transformed to a logarithmic scale and all computations made on the transformed figures This was accordingly also done in testing the reproducibility of the technique.

The reproducibility of the technique was calculated for each skin area in 3 groups of 5 subjects. Tested by an analysis of

Table 2 *Per cent of test bacteria recovered from fingers of 15 individuals*

No of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11 9		19 7	
14 1		39.5	
12.2	12 4	34 1	39 0
11.9		56.3	
12.1		45.2	
4.6		66.7	
4 4		32 7	
4.8	4.5	40 0	40.5
4.2		3 4	
4.5		25.8	
9 4		50.7	
8.3		60.8	
8.6	9 1	18.1	40.3
9 4		23 4	
9 6		48.5	

Table 5. *Per cent of test bacteria recovered from axillae of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 5 suspensions	Mean	3 groups of of 5 individuals	Mean
9.4		28.7	
8.5		11.5	
8.6	9.1	9.7	19.0
9.4		28.7	
9.6		16.5	
16.7		30.5	
15.9		40.0	
15.9	16.9	9.2	21.9
17.1		19.4	
19.0		10.8	
1.8		42.2	
1.7		34.1	
2.0	1.8	11.4	26.4
1.5		9.1	
2.0		25.0	

homogeneity of variance according to M. S. Bartletts formula (119) for approximate χ^2-distribution, the group variances did not prove to be significantly different ($P > 5$ per cent) The mean values for each group were then tested by an ordinary one-way analysis of variance. The calculated F values with $f_1=2$ and $f_2=12$ degrees of freedom were 0.7121 for upper lip, 0.2238 for fingers, 0.2612 for axillae, 1.8126 for skin of abdomen 0.2727 for perineum and 0.1195 for hands. The corresponding P-values were 20 per cent for the skin of abdomen and more than 50 per cent for the other skin areas. Consequently there was no significant difference between the groups. Thus, the reproducibility of the methods was considered to be satisfactory

The number of staphylococci lost during treatment of the swabs was investigated in the following manner Five swabs were infected with 0.1 ml of a suspension of staphylococci and treated in physiological saline as previously described. Three such experiments were performed and the number of viable organisms was calculated for each experiment in five 0.1 ml samples of the suspension used The results are given in table 7 From 50 to almost 100 per cent of the infecting organisms were recovered from the swabs.

From patients who were staphylococcal skin carriers, three consecutive samples were obtained from the same skin site, using swabs in the manner previously described. The results are given in table 8. The first sample always yielded the ma

Table 4 *Per cent of test bacteria recovered from skin of abdomen of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 5 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		66.6	
14.1		58.2	
12.2	12.4	44.4	57.9
11.9		63.6	
12.1		55.7	
4.6		52.5	
4.4		40.9	
4.8	4.5	49.8	54.4
4.2		56.9	
4.5		71.1	
11.7		36.4	
15.2		73.6	
13.4	13.0	42.0	57.5
12.0		71.4	
14.9		64.1	

Table 1 *Per cent of test bacteria recovered from upper lip of 15 individuals*

No of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		46.3	
14 1		29.3	
12.2	12 4	63 7	49.3
11.9		50.5	
12 1		56.6	
4 6		24 0	
4 4		58.7	
4 8	4 5	42 7	40.6
4.2		21 4	
4 5		56 0	
9 4		57.3	
8.3		60.8	
8 6	9 1	18.1	44.3
9 4		56.8	
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nose, throat upper lip axillae, skin of abdomen and perineum) the described areas of upper lip 1st and 2nd fingers of one hand skin of abdomen, axillae and perineum were infected with 0 04 ml and the hands with 1 ml of a suspension of Staph aureus in physiological saline. For each skin area 3 groups of 5 subjects were infected, and for each group the numbers of infecting organisms were calculated in five samples of the suspension used.

After the skin had dried for 5 minutes, samples were obtained by the methods described The results are given in tables 1—6 Within each group the variations in the numbers of organisms used for infection were small but the numbers of organisms isolated from the different subjects varied to a much greater extent. However compared with the results of the later investigations (chapters IV V VI and VII) these variations were of minor importance.

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The reproducibility of the technique was calculated for each skin area in 3 groups of 5 subjects. Tested by an analysis of

Table 2 *Per cent of test bacteria recovered from fingers of 15 individuals*

No of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		19 7	
14 1		39.5	
12.2	12 4	34 1	39 0
11.9		56.3	
12 1		45.2	
4 6		66.7	
4 4		32.7	
4.8	4.5	40.0	40.5
4.2		37 4	
4.5		25 8	
9.4		50 7	
8.3		60.8	
8 6	9 1	18.1	40.3
9 4		23 4	
9 6		48.5	

Table 7 *Per cent of test bacteria recovered from 15 swabs*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of 5 swabs	Mean
1.8		60.3	
1.7		69.0	
1.8	1.9	81.9	73.7
2.1		94.8	
1.8		62.5	
1.3		99.5	
1.8		89.0	
1.3	1.5	89 0	79.6
1.6		49 7	
1.6		70.7	
10.6		81.5	
9.7		72.5	
9.5	9.8	78.6	81.2
9 0		90.9	
10.4		82.3	

Scrubbing the hands for two minutes removes the transient flora (103). In the present investigation (table 9) the transient flora of the hands (as well as some of the resident flora) should consequently have been removed by the three hand washings. Since 19 to 66 per cent of the total number of Staph. aureus in the 3 hand washings was demonstrated in the first bowl the results obtained by the standardized hand washing should be representative of the number of staphylococci in the transient flora of the hands.

The considerable drop in the number of staphylococci from the 2nd to the 3rd hand washing supports the theory that most of these organisms belong to the transient skin flora. Accordingly the results obtained by this method should also be representative of the total number of Staph. aureus on the hands of most of the skin carriers.

Table 9 *Number of staphylococci in 3 consecutive samples from the hands of 10 skin carriers. No. of colonies × ml. (in thousands)*

Pat. no.	Sample no.			Total	1st sample as per cent of total
	1	2	3		
1	182.0	82.0	14.0	278.0	65.5
2	145.0	75.0	3.5	223.5	64.9
3	36.0	48.0	7.0	91.0	39.6
4	25.0	36 0	2.5	63.5	39.4
5	39.0	41.5	3.5	84.0	46.4
6	90.0	100.0	10.0	200.0	45.0
7	305.0	285.0	61.0	651.0	46.9
8	90 0	80.0	9.5	179.5	50.1
9	110.0	370.0	100.0	580.0	19.1
10	840.0	1,360.0	46.0	2,246.0	37 4

Table 5 *Per cent of test bacteria recovered from perineum of 15 individuals*

No of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of 5 individuals	Mean
145 0		58.5	
131 0		50.9	
150 0	145 4	44 7	42.6
144 0		50.2	
157 0		28 9	
15 4		-0 1	
15.5		22.3	
15 0	15.9	57 7	42.8
16.3		48.4	
14 6		55.5	
12.9		25.3	
14 5		34 9	
13 7	13.9	74 7	48.7
15 4		30.5	
13 1		56.9	

jority of the staphylococci only small numbers being isolated in the last sample.

To investigate whether the results obtained by the hand washing technique were representative of the actual number of staphylococci on the hands 10 patients who were staphylococcal skin carriers performed the following experiment. After the standardized hand washing they scrubbed their hands for two minutes in a second bowl of water rinsed them thoroughly in this water and scrubbed their hands again for two minutes in a third bowl Counts of the viable staphylococci in each bowl were made. The results are given in table 9 The ratio between the numbers of staphylococci in the first bowl, and in the three bowls together varied between 19 and 66 per cent. There was a significant decrease in the number of organisms from the 2nd to the 3rd hand washing

d Discussion

In consecutive samples obtained from the same skin site, using moistened swabs, the majority of Staph. aureus were demonstrated in the first sample and relatively small quantities or nothing at all in the last test. Compared with the results obtained in experiments using test organisms these investigations show that the numbers of staphylococci demonstrated by the method described were representative of the quantities of these organisms in the transient flora where they usually appear (81 82 103 104)

Table 6 *Per cent of test bacteria recovered from hands of 15 individuals*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
188		23 4	
150		24 7	
160	16?	49 4	42.0
168		55.6	
146		56.8	
608		43 4	
618		26.2	
606	649	21 0	33 0
692		30.2	
720		44 1	
646		37.8	
722		41 1	
654	661	33.6	32.2
628		46.9	
656		16 0	

B. Method of quantitative estimation of Staph. aureus in the vestibule of the nose.

1. *Previous methods.*

Nasal cultures have usually been obtained from the vestibule with cotton wool swabs and spread on various solid media or put into broth. Some authors (123) claimed that they achieved a higher frequency of positive samples by so-called deep swabbing i.e. obtaining samples from the vestibule and backwards to below the middle concha. On this point, however opinions are divided. Williams et al. (149) maintained that nothing would be gained by swabbing the higher reaches of the nose, and in fact, that mistakenly swabbing beyond the vestibule might produce artificially low carrier rates. The investigations of Moss et al. (98) support this view

The quantitative assessment of these organisms in the nasal vestibule has generally been based on a rather crude distribution into three groups (scanty moderate and abundant) of staphylococcal colonies which grew on solid media stroked by the culture stick. Siboni (117) used a more differentiated distribution into 6 groups. Others (55 56, 107) have counted those plates which contained less than 1,000 colonies, while those yielding more were grouped as ∞.

White et al. (142) obtained nasal cultures by means of cotton wool swabs moistened in broth. The swabs were put into small tubes containing 3 ml broth and shaken for 5 minutes in a Khan shaker Serial ten-fold dilutions of the broth were then plated on agar media. After incubation, the pigmented colonies were counted and multiplied by the dilution factor

Presumably the last method gave the best expression of the number of Staph. aureus in the nasal vestibule

2. *Personal method.*

a. Description.

Nasal samples were obtained from the entire anterior nares, both lateral and medial walls. First a cotton wool swab (the cotton wool covered 2—3 cm of the end of the swab) moistened in 0.9 per cent saline was rubbed with even pressure and constant rotation five times round the inside of each nostril parallel to the skin. Immediately afterwards the anterior nares were examined in the same way but this time using a dry swab. The swabs were treated in saline as described for skin samples and the number of staphylococci calculated.

b. Reproducibility

It was difficult to implant known quantity of Staph. aureus in the nasal vestibule. The reproducibility of the technique was therefore tested by obtaining 10 consecutive daily nasal cultures from 4 individuals, who had been shown by 6 samples taken at 3-day intervals to be persistent

Table 10. *Number of staphylococci in daily nasal samples from 4 persistent nasal carriers No. of colonies × dil. (in thousands)*

Day no.	Individ. no.				Mean
	1	2	3	4	
1	168	112	212	3,920	1 103
2	856	124	172	920	518
3	160	40	80	6,120	1,600
4	252	104	408	1,960	681
5	160	284	364	4,320	1,282
6	296	324	524	880	506
7	100	76	196	2,240	653
8	1,040	136	280	760	554
9	440	48	764	6,040	1,823
10	960	40	728	4,600	1,582
Mean	443	129	373	3,176	(1,030)

Table 8 *Number of staphylococci in 3 consecutive samples from the same skin area. No. of colonies × dil. (in thousands)*

Pat. no.	Skin area	Sample no. 1	2	3	Total (range)	1st sample as per cent of total (mean)
1	Fingers	0.34	0 02	<0 02	0.38—0.36	91.9
2		0 70	0 16	<0 02	0.88—0.86	80.5
3		1.92	<0 02	<0 02	1.96—1.92	99.0
4		0 08	<0 02	<0 02	0 12—0.08	80.0
5		0 04	<0 02	<0 02	0 08—0.04	66.7
6		105.00	20 00	1 00	126 00	83.3
7		0.88	0 18	0.06	1 12	78.6
8		20.00	3.00	0.28	23.28	85.9
9		0 40	0.10	0 02	0.52	76.9
10		85 00	15.00	0.20	100.00	85.0
11		52.00	9 00	1 00	62.00	83.9
12	Upper lip	0 08	0.02	<0 02	0.12—0 08	80.0
13		1 08	0 12	<0 02	1.22—1.20	89.5
14	Axillae	168 00	120.00	11 00	299 00	56.2
15		680 00	284 00	32.00	996 00	68.3
16	Perineum	1 60	0 44	0 16	2.20	72 7
17		244 00	96 00	4 00	344 00	70.9
18		32 00	7.60	2.00	41.60	6.9
19		56.00	2.80	0.80	59 60	94 0
20		2 08	0 44	0.08	2 60	80.0
21		27 480.00	6,780.00	200.00	34 460 00	79.8
22	Dermal lesion	38,700 00	18 300 00	6,360.00	63,360 00	61 1
23		4,960.00	3,980.00	1 750 00	10 690.00	46.4
24		91 00	27 00	4.00	122 00	74 6
25		44,800 00	13 600.00	6,000.00	64 400 00	69.6

c. *Summary and conclusions.*

1 A method of quantitative estimation of Staph. aureus on different skin areas and a method of determining staphylococcal contamination of the hands are described

2. By infecting different skin areas with a suspension of Staph. aureus it was demonstrated that the reproducibility of the methods was satisfactory

3 The results obtained by the methods were representative of the number of Staph aureus in the transient skin flora of staphylococcal carriers.

c. Discussion.

The variations demonstrated in the nasal counts from the 4 individuals (table 10) comprised both variation in the actual number of nasal organisms from day to day and variation in the technique. If a constant number of staphylococci in the nose had been maintained from day to day the changes demonstrated would have expressed only variation in the technique. However the probability that some of the variations were due to changes in the number of organisms in the nasal vestibule, would indicate that the technique was better than demonstrated in this investigation.

Obtaining several consecutive nasal cultures from the same individual should have eliminated the natural variations in the quantity of nasal staphylococci. If the results given by the method described were representative of the number of staphylococci in the nose, the quantitative variations for nasal carriers from the first to the last sample should be approximately parallel. This was demonstrated by means of 5 consecutive nasal cultures from 10 nasal carriers.

d. Summary and conclusion.

1. A method of evaluating the number of Staph. aureus in the nasal vestibule is described.

2. Experiments with persistent nasal carriers of staphylococci showed that, within wide limits, the results obtained by this method were representative of the number of these organisms in the nasal vestibule.

C. Method of quantitative evaluation of Staph. aureus in the throat.

Throat samples were obtained by dry cotton wool swabs which were rubbed twice over both tonsils and the soft palate and treated in physiological saline as previously described. The number of staphylococci was calculated as described for skin samples.

D. Method of quantitative evaluation of Staph. aureus in the vagina.

Vaginal samples were obtained by dry cotton wool swabs which were rubbed once over the mucous membrane of the vagina. After treatment in physiological saline, 1/1,000 and 1/40 000 of the suspension were incubated on mannitol salt agar and the number of staphylococci calculated.

E. Method of quantitative evaluation of Staph. aureus in the faeces.

1 g of faeces (taken just after defaecation) was collected with a dry swab. The faeces were stirred into physiological saline 1/1 000 and 1/40 000 of the suspension incubated on mannitol salt agar and the number of staphylococci calculated.

F. Assessment of the ability of individuals to disperse Staph. aureus into the air.

1. *Previous methods.*

It is generally accepted that staphylococcal carriers disperse their organisms into the air mainly on desquamated skin scales (29) from their skin and clothes (31, 54). Direct investigation of the staphylococcal contamination of the patients' clothes would therefore give an indication of their ability to contaminate the air with these organisms. However, a more accurate picture would be obtained by direct measurement of the air contamination caused by bed making, dressing and un-

nasal carriers (the method of selection was the same as for nasal carriers in chapter IV) The results are given in table 10 The reproducibility of the technique was tested by an analysis of homogeneity of variance according to M. S Bartlett's formula (119) for approximate χ^2-distribution The calculated value for χ^2 was 0 433 with three degrees of freedom, and the corresponding P value was 93 per cent. Consequently the reproducibility of the technique was considered to be satisfactory

Five consecutive (immediately after each other) nasal cultures were obtained from 10 patients who were persistent nasal carriers on 6 examinations at 3-day intervals. The numbers of staphylococci in the nasal samples are given in table 11 and the logarithms of the cumulative values in fig 1 The first two samples yielded considerably larger numbers of organisms than the last two and the numbers for the 10 nasal carriers decreased approximately in parallel from the first to the last sample.

Table 11 *Number of staphylococci in 5 consecutive nasal samples from 10 persistent nasal carriers No of colonies × dil. (in thousands)*

Pat. no	Sample no.				
	1	2	3	4	5
1	640	960	480	480	120
2	120	40	48	8	4
3	2,880	2,000	840	520	440
4	2 480	1 760	1,320	480	360
5	1 040	680	160	200	120
6	1 040	1 040	480	360	280
7	12 080	7,920	4,200	320	200
8	1,560	1,920	280	160	120
9	5 680	4 440	560	600	760
10	524	244	124	84	40
Mean	2,804	2 100	849	371	244

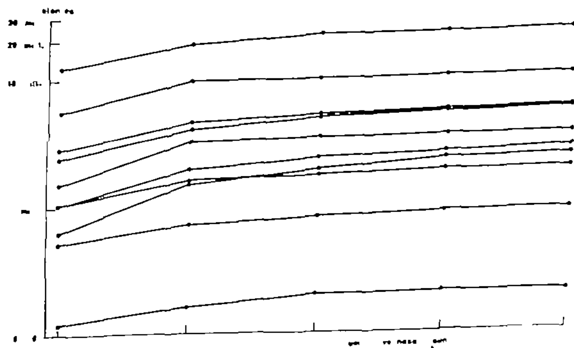


Fig 1 Cumulative staphylococcal counts in 5 consecutive nasal cultures from 10 nasal carriers.

c. Discussion.

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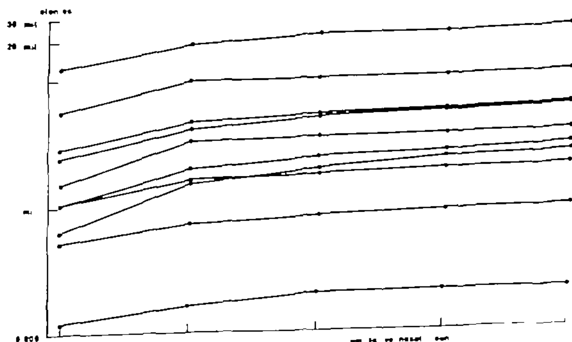


Fig 1 Cumulative *staphylococcal* counts in 5 consecutive nasal cultures from 10 nasal carriers.

a small room (the air-lock) 1.90 m high, 1.05 m long and 0.28 m wide. The plastic wall could be opened by means of a zip. A slit-sampler was connected with the test room by a 40 cm long tube through the wall. The floor was covered with linoleum and the walls and ceiling were lacquered.

After examining 120 c. ft. of the air of the test room with the slit-sampler to ensure that it was not contaminated by staphylococci, the bed was moved into the room and placed as shown in fig. 2. The air-holes and the air-lock were closed and 10 sedimentation plates were disposed on the floor and the bed as shown in fig. 2. The slit-sampler was set in action and, at the same time, a nurse began to make the bed. The eiderdown was folded up and placed over the foot of the bed. The pillows were laid on top of the eiderdown. The sheets were brushed with the hand from the middle of the bed to both sides. The draw-sheet, which lay across the bed, was loosened on one side and shaken twice. Both sheets were secured under the mattress. The pillow and the eiderdown were shaken 3 times, half a metre above the top of the bed, and put back on the bed.

All the beds were made by the same nurse. On repeated examinations of her skin and clothes before she went into the test room, no staphylococci were isolated. The complete bed making lasted 3 $\frac{1}{2}$ minutes. The slit-sampler collected 24 c. ft. of air per minute for 5 minutes. When it was finished, the nurse let herself out through the air-lock. After the bed had been in the test room for an hour the air-lock and the air-holes were opened, the sedimentation plates were closed and the bed removed.

After incubation of the plates for 48 hours at 37°C the staphylococcal colonies were counted. The 10 sedimentation plates covered 1/100 of the floor of the test room (the surface area of 10 plates was 0.058 sq.m, the floor area being 5.8 sq m). The total air contamination was calculated by multiplying the number of staphylococcal colonies on the sedimentation plates by 100.

In preliminary experiments (see table 15) it was shown that the number of staphylococcal colonies on the slit-sampler plate was, on average, four times as great as the number on the 10 sedimentation plates. If no staphylococcal colonies were demonstrated on the sedimentation plates, the air contamination was calculated by multiplying the number of colonies on the slit-sampler plate by 25. When there was no growth of Staph. aureus on any of the plates the number of colonies was reported as less than 25 in the tables. However, in statistical calculations two alternative values were used, namely 24 and 1, the maximum and the minimum number less than 25 (1 is used instead of 0 as the calculations were based on the logarithms of the numbers. $\log_{10} 1 = 0$, $\log_{10} 0 = -\infty$)

Before the next examination the test room was aired for half an hour and the floor washed twice with a quarter of an hour interval. The walls and the ceiling were washed every third day with ordinary soap and water.

b. Reproducibility

Exposure time and number of sedimentation plates. The sedimentation plates were exposed to air contamination for one hour. The question of whether this was adequate time for the greater part of the bacteria-carrying particles to sediment, was investigated in the following experiment. The bed of a heavy staphylococcal disperser was made as previously described. Continuous samples were obtained with the slit-sampler (1 c.ft. per minute) for 36 minutes. Twenty-five minutes later a

dressing etc — activities which are known to cause a major part of the bacterial air contamination in *hospitals* (10 57 73 87 99)

Several methods have been employed for quantitative estimation of the bacterial contamination of textiles Loosli et al (80) used a slit-sampler as a vacuum-cleaning device and measured the number of organisms removed. Williams 'sweep-plate method (12) has frequently been used In the percussion method (96) a weight is dropped onto a piece of cloth stretched over a culture plate. The contact plate method (112) rinsing (50) and maceration (115) of a piece of cloth in a suitable fluid are also well known methods.

Air contamination caused *directly* by staphylococcal carriers in the performance of everyday activities e.g dressing undressing walking standing still talking coughing etc. has been measured only in a few investigations Duguid and Wallace (31) investigated air contamination from clothing by letting 4 individuals perform certain gymnastic exercises dress and undress and brush their clothes in a small room of 100 c. ft The air contamination *was measured by a slit sampler*

Hare and Thomas (54) used a rather smaller cubicle In this room, the clothes of test subjects were hung up and shaken by means of cords attached to the elbows of the jacket and knees of the trousers. In other experiments the subjects were allowed to mark time and swing their arms for 15 minutes. Air contamination was measured by *three sedimentation* plates at different heights in each corner of the room

Hare and Ridley (55) used a tubular plastic cubicle hanging from a hoop It reached to the shoulders of the subjects so that the head protruded through an opening in the roof" Air contamination was measured by 4 sedimentation plates. Dressed in their everyday clothes the individuals performed the same gymnastic exercises as in the experiments of Hare and Thomas (54)

White (144) investigated the air contamination in the neighbourhood of the patients beds, using slit-samplers He tried to observe the conditions generally present in the ward and no attempts were made to increase or diminish activity during the investigation period

2. *Personal method*

In the present study the staphylococcal air contamination caused by a standardized *form of bed making in a test room has been* investigated

a. Description

Fig 2 shows a drawing of the test room. A plastic wall was put up in front of the door This and the door together formed

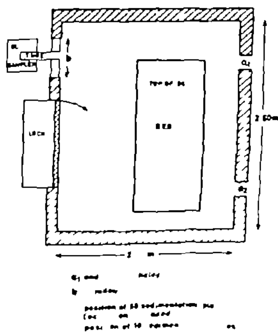


Fig 2. Test room topography

together with the total air contamination calculated on the basis of 10 and 50 plates. The difference in the air contamination calculated from 10 and 50 plates was small and did not justify the use of more than 10 plates.

The reproducibility of the nurse's bed making technique. Air contamination was investigated in a series of preliminary experiments in the test room where six nurses made beds which were artificially contaminated with equal quantities of staphylococcal particles. The mean value for each experiment was calculated and one of the nurses was given the task of practising bed making with and without patients, so that air contamination was approximately that of the mean values demonstrated. She subsequently made all the beds in the present investigation.

The following test of the reproducibility of her technique was performed. The same areas of the sheets, eiderdown and pillows in six clean beds were contaminated with equal quantities of staphylococcal particles one hour before the beds were made. There was a patient in only one bed (No. 2). Five such experiments were undertaken. The quantity of organisms and the size of the areas of sheets, eiderdowns and pillows which were contaminated varied from experiment to experiment. The air contamination was measured by 10 sedimentation plates. The results are given in table 13. The variations in air contamination within each experiment were small. The reproducibility of the technique was tested by an analysis of homogeneity of variance based on the logarithms of the observations. It was assumed that the technique was reproducible if there were no difference on the 5 per cent level of significance for each experiment, tested according to M. S. Bartlett' formula for approximate χ^2-distribution. The

Table 13. *Degree of air contamination in test room caused by making artificially contaminated beds. No. of bacterial particles (in thousands)*

Bed no.	Exp. no.					Mean
	1	2	3	4	5	
1	7.9	1.4	2.2	1.9	6.6	4.0
2	4.9	0.7	1.8	1.7	8.4	3.5
3	5.3	0.9	1.9	1.5	6.1	3.1
4	4.7	0.9	2.3	1.1	6.8	3.2
5	7.7	0.6	2.	1.5	5.4	3.6
6	11.9	1.2	1.1	1.2	4.3	3.9
Mean	7.1	1.0	2.0	1.5	6.3	(3.6)

calculated value for χ^2 was 2.5385 with four degrees of freedom and the corresponding P-value was 80 per cent. Consequently the reproducibility of the technique was considered to be satisfactory.

The relationship between the number of Staph. aureus particles demonstrated on 10 sedimentation plates in the test room and a large room in the department. Two patients had to be examined in a large room (No. 19) in the department because the number of staphylococcal colonies on the sedimentation plates put out in the test room was too large to be counted with accuracy. Room 19 was 4.5 × 4.5 m. In this room, the plates could be placed further from the beds than in the test room.

The relationship between the degree of air contamination demonstrated in the two rooms was investigated by contaminating four beds with equal quantities of staphylococcal particles. Two beds were made in the test room and two in room 19. The results of four such experiments are shown in table 14. Calculated according to the mean values for all experiments, the ratio between the number of colonies demonstrated in the test room and in room 19 was 4.2 : 1. The air contamination by

second sample was obtained for five minutes One hour after the bed making had commenced the sedimentation plates were removed and new ones put out for 10 hours. Four such experiments were performed As parallel results were obtained the results of only one experiment are given in fig 3 Staphylococcal air contamination

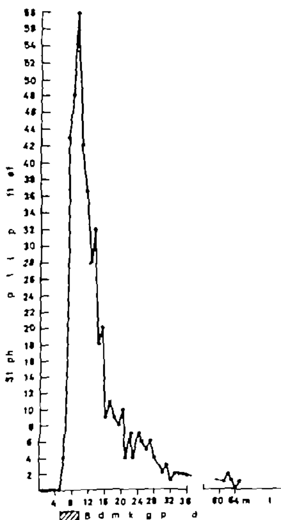


Fig 3 Air contamination in test room caused by making the bed of a heavy staphylococcal disperser

was far greater during and just after bed making than 1 hour later On 10 sedimentation plates exposed for one hour from the commencement of bed making 1,328 staphylococcal colonies were counted while only 23 colonies were counted on the 10 new plates put out for the next ten hours Similar results have been obtained in earlier investigations in the same room (18) Thus one hour's exposure of the sedimentation plates was sufficient to demonstrate the greater part of the air contamination due to bed making

To ensure that 10 sedimentation plates were sufficient to give an approximately correct picture of the air contamination the following experiment was undertaken A few beds were made as previously described The staphylococcal air contamination was measured by 50 sedimentation plates evenly distributed over the bed and floor as shown in fig 2. The plates on the bed were disposed on stands in order not to impede the bed making Ten of the 50 distributed plates were placed at the same sites as those in the later investigations. Eight such experiments were performed Table 12 shows the number of staphylococcal colonies on 10 versus 50 plates

Table 12 *Degree of air contamination in test room judged from the number of colonies (in hundreds) on 10 versus 50 sedimentation plates*

Exp. no.	No. of cols. on		Total air contamination determined from	
	10 plates	50 plates	10 plates	50 plates
1	0.21	0.96	21.0	19.2
2	0 18	1.25	18.0	25 0
3	0.58	3.23	58.0	64 6
4	1 42	7.07	142 0	141 4
5	0.86	3.89	86.0	77.8
6	0.40	2 17	40.0	43.4
7	1.04	5 49	104.0	109.8
8	1.34	6.27	134.0	125 4
Mean	0 75	3 79	75.4	75.8

2. By making artifically contaminated beds it was demonstrated that the re producibility of the technique was good.

G Bacteriological technique.

The samples were plated on mannitol salt agar medium. After incubation for 48 hours at 37°C the staphylococcal colonies were counted. Five to ten colonies from the air samples, and 1—3 generally 2, colonies from the other samples, were plated on blood agar After incubation of the blood agar plates for 18 hours at 37°C the subcultures were examined for coa gulase production. All coagulase positive colonies were transferred to broth and antibiogram determinations and phage typing were carried out. If antibiograms and phage patterns left any doubt as to whether tw strains from the same individual were identical, serological typing was performed.

1 *Culture media.*

The mannitol salt agar medium was d scribed by Chapman (22) This medium inhibits the growth of gram-negative rods and B. subtilis (21 22, 38) and the staphy lococcal colonies are well coloured (21)

The degree of accuracy by which the Staph. aureus colonies were distinguished from other bacterial colonies on this me dium was investigated in the following manner The staphylococcal colonies and other bacterial colonies were counted on 10 mannitol salt agar plates which con tained samples of skin and air contamina tion from 5 patients. Fifty-seven definite and 3 uncertain Staph. ureus colonies were counted and 334 colonies of other bacteria. All colonies were submitted to further examinations (acid and coagulase production, microscopic examinations etc.) The first group of 57 colonies were all coagulase positive staphylococci and the other 337 colonies were found to be other bacteria.

In the later experiments, the same classification of the colonies was undertaken. All uncertain colonies were examined for coagulase production. In consequence the number of wrongly diagnosed colonies should be small and of minor importance in the results of the present investigation. The blood agar medium was composed of

1.8 per cent agar Kobe.

1.0 per cent peptone, Witte.

0.5 per cent sodium chloride (May and Baker)

7 0 per cent human blood.

Meat extract (0.5 kg beef in 1,000 ml water) ad 1,000 ml. ph adjusted to 7.5

This was used as a subculture medium to void mannitol fermentation products which might impede the coagulase test (15 131)

2. *The coagulase test.*

To rabbit serum stabilized with sodium citrate and diluted 1 5 with broth, a loop of blood agar culture was added, shaken and incubated at 37°C Each tube contained 1 ml fluid. After 3 4 and 24 hours the results were recorded. Negative tests were examined twice. The capacity to coagulate plasma has been used as the only criterion for the selection of pathogenic staphylococci.

3 *Antibiogram determinations.*

The disc method (36, 128) was employed to determine the sensitivity to sulphathiazole, penicillin, streptomycin, tetracycline, erythromycin and chloramphenicol, using sensitivity discs manufactured by Klinisk-bakteriologiska Laboratorier Karolinska sjukhuset, Stockholm.

Three to five ml of a broth suspension of Staph. aureus suitable for producing discrete colonies was transferred to peptone-free blood agar plates containing 1

Table 14 *Degree of air contamination caused by making artificially contaminated beds in the test room versus a patient room.*
No of colonies on 10 sedimentation plates

Exp. no	Test room			Patient room		
	Bed		Mean	Bed		Mean
	1	2		1	2	
1	66	54	60	20	11	15.5
2	68	54	61	13	10	11.5
3	33	36	34.5	7	12	9.5
4	49	87	68	21	13	17.0
Total	216	231	223.5	61	46	53.5

the two patients who were examined in room 19 was calculated by multiplying the number of staphylococcal colonies on the plates by 4 2 × 100

The relationship between the number of Staph. aureus particles demonstrated by the slit sampler and by the sedimentation plates Estimation of the number of staphylococcal colonies on the slit sampler plate was difficult when the air contamination was heavy The number of colonies on the sedimentation plates was therefore used as a basis for the calculation of the air contamination. However when no colonies were demonstrated on the sedimentation plates, the number on the slit-sampler plate formed the basis of the calculation. The relationship between the number of colonies demonstrated by the slit-sampler and by the sedimentation plates was calculated in 10 experiments. The results are shown in table 15 On the average, four times (precisely 3 95) as many staphylococcal colonies were demonstrated by the slit sampler (24 c.ft per minute for 5 minutes) as by the 10 sedimentation plates

c. Discussion.

The air contamination produced by making equally contaminated beds varied little. This is in agreement with investigations undertaken by the percussion method (114) In principle, the technique used in the present study was similar to the percussion method Hare and Ridley (55) found it difficult to make the test subjects use a uniform degree of activity while exercising in the cubicle. In the present study this difficulty was avoided by letting the same nurse make all the beds.

The number of organisms demonstrated in air samples decreases with the square of the distance from the bacterial spreading process. The plates and beds were therefore placed in fixed positions, so that the distance should be constant in all experiments

d Summary and conclusion

1 To assess the ability of individuals to disperse staphylococci into the air a standardized form of bed making in a test room is described The air contamination was measured by sedimentation plates and a slit-sampler

Table 15 *Degree of air contamination in test room. Slit-sampler (24 c ft./min for 5 min.) versus 10 sedimentation plates (exposed for 1 hour)*
N of colonies

Exp no.	Slit-sampler A	Sed.plates B	Ratio $\frac{A}{B}$
1	19	5	3 8
2	60	13	4 6
3	49	12	4 1
4	87	24	3.6
5	66	20	3.3
6	9	2	4.5
7	11	3	3 7
8	13	3	4.3
9	23	4	5.8
10	26	6	4.3
Mean	36.3	9.2	4 0

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G Bacteriological technique.

The samples were plated on mannitol salt agar medium. After incubation for 48 hours t 37°C the staphylococcal colonies were counted Five to ten colonies from the air samples, and 1—3 generally 2, colonies from the other samples, were plated on blood agar After incubation of the blood agar plates for 18 hours at 37 C the subcultures were examined for coagulase production. All coagulase positive colonies were transferred to broth and antibiogram determinations and phage typing were carried out. If antibiograms and phage patterns left any doubt as to whether two strains from the same individual were identical, serological typing was performed

1 *Culture media.*

The mannitol salt agar medium was de scribed by Chapman (22) This medium inhibits the growth of gram-negative rods and B. subtilis (21 22, 38) and the staphylococcal colonies are well coloured (21)

The degree of accuracy by which the Staph. aureus colonies were distinguished from other bacterial colonies on this medium was investigated in the following manner The staphylococcal colonies and other bacterial colonies were counted on 10 mannitol salt agar plates which contained samples of skin and air contamina tion from 5 patients. Fifty-seven definite and 3 uncertain Staph. aureus colonies were counted, and 334 colonies of other bacteria. All colonies were submitted to further examinations (cid and coagulase production microscopic examinations etc.) The first group of 57 colonies were all coagulase positive staphylococci and the other 337 colonies were found to be other bacteria.

In the later experiments, the same classification of the colonies was undertaken All uncertain colonies were examined for coagulase production. In consequence the number of wrongly diagnosed colonies should be small and of minor importance in the results of the present investigation.

The blood agar medium was composed of

1.8 per cent agar Kobe

1 0 per cent peptone W tte.

0.5 per cent sodium chloride (May and Baker)

7 0 per cent human blood

Meat extract (0.5 kg beef in 1,000 ml water) ad 1,000 ml. ph adjusted to 7.5

This was used as a subculture medium to avoid mannitol fermentation products which might impede the coagulase test (15 131)

2. *The coagulase test*

To rabbit serum stabilized w th sodium citrate and diluted 1 5 with broth, a loop of blood agar culture was added, shaken and incubated at 37°C Each tube contained 1 ml fluid. After 3 4 and 24 hours the results were recorded. Negative tests were examined twice. Th capacity to coagulate plasma has been used as the only criterion for the selection of pathogenic taphylococci.

3 *Antibiogram determinations.*

The disc method (36, 128) was employed to determine the sensitivity to sulphathiazole penicillin, streptomycin, tetra cycline erythromycin and chloramphenicol using sensitivity discs manufactured by Klinisk-bakteriologiska Laboratoriet, Karolinska sjukhuset, Stockholm.

Three to five ml of a broth suspension of Staph. aureus suitable for producing discrete colonies was transferred to peptone-free blood agar plates containing 1

Table 14 *Degree of air contamination caused by making artificially contaminated beds in the test room versus a patient room*
No of colonies on 10 sedimentation plates

Exp no.	Test room			Patient room		
	Bed		Mean	Bed		Mean
	1	2		1	2	
1	66	54	60	20	11	15.5
2	68	54	61	13	10	11.5
3	33	36	34.5	7	12	9.5
4	49	87	68	21	13	17 0
Total	216	231	223.5	61	46	53.5

the two patients who were examined in room 19 was calculated by multiplying the number of staphylococcal colonies on the plates by 4.2 × 100

The relationship between the number of Staph. aureus particles demonstrated by the slit sampler and by the sedimentation plates Estimation of the number of staphylococcal colonies on the slit-sampler plate was difficult when the air contamination was heavy The number of colonies on the sedimentation plates was therefore used as a basis for the calculation of the air contamination However when no colonies were demonstrated on the sedimentation plates the number on the slit-sampler plate formed the basis of the calculation The relationship between the number of colonies demonstrated by the slit-sampler and by the sedimentation plates was calculated in 10 experiments. The results are shown in table 15 On the average four times (precisely 3 95) as many staphylococcal colonies were demonstrated by the slit sampler (24 c.ft. per minute for 5 minutes) as by the 10 sedimentation plates.

c. Discussion

The air contamination produced by making equally contaminated beds varied little. This is in agreement with investigations undertaken by the percussion method (114) In principle the technique used in the present study was similar to the percussion method Hare and Ridley (55) found it difficult to make the test subjects use a uniform degree of activity while exercising in the cubicle. In the present study this difficulty was avoided by letting the same nurse make all the beds.

The number of organisms demonstrated in air samples decreases with the square of the distance from the bacterial spreading process. The plates and beds were therefore placed in fixed positions, so that the distance should be constant in all experiments.

d Summary and conclusion

1 To assess the ability of individuals to disperse staphylococci into the air a standardized form of bed making in a test room is described The air contamination was measured by sedimentation plates and a slit-sampler

Table 15. *Degree of air contamination in test room. Slit sampler (24 ft./min. for 5 min.) versus 10 sedimentation plates (exposed for 1 hour)*
No of colonies

Exp. no.	Slit-sampler *A*	Sed.plates *B*	Ratio $\frac{A}{B}$
1	19	5	3.8
2	60	13	4.6
3	49	12	4 1
4	87	24	3.6
5	66	20	3 3
6	9	2	4 5
7	11	3	3 7
8	13	3	4.3
9	23	4	5.8
10	26	6	4.3
Mean	36.3	9.2	4.0

Two cultures were considered to be different when one was lysed strongly by at least two phages which produced no degree of lysis of the other (2, 11, 146). Sometimes (about 1 per cent) subcultures from the same colony differ by more than one "strong" reaction (146). In the present study strains which were presumed to be identical, e.g. demonstrated in the nose and skin samples from the same individual, occasionally differed by two "strong" reactions, while the antibiograms were identical. In such cases several colonies (5—15) from the samples were phage typed. If there were still doubt whether the original strains were identical, serological typing was performed.

The phage typing was performed at The Gade Institute, Department of Microbiology.

5. *Serological typing*

Serological typing was performed according to the technique described by Oeding (100). Factor sera *m* (59), *n* (59), (59), *a* (60), h_3 (62), *c* (61), 263—1 (67) and 263—2 (67) were employed. Two strains were considered to be different if they differed in one or more antigens.

The serological typing was performed at The Gade Institute, Department of Microbiology.

Table 16 *Ratio between inhibition zone diameter and sensitivity to antimicrobial agents*

Degree of sensitivity	Inhibition zone (range mm)					
	Sulpha thiazole	Penicil lin	Erythro-mycin	Strepto-mycin	Chloram-phenicol	Tetra cycline
Highly sensitive	29—35	31—44	26—46	20—35	22—44	21—38
Fairly sensitive	*23—28*	*21—30*	*20—25*	*16—19*	*16—21*	*17—20*
Relative *resistant*	*17—22*	*12—20*	*12—19*	*12—15*	*11—15*	*9—16*
Resistant	5—16	5—11	5—11	5—11	5—10	5— 8

per cent glucose The plates were first de canted and the excess fluid pipetted off and then dried horizontally for 30 minutes at 37 C The discs were placed aseptically on the substrate at least 4 cm apart The plates were incubated for 18—20 hours at 37°C and the diameter of the inhibition zone was measured The sensitivity was divided into (1) highly and (2) fairly sensi tive groups and in (3) relatively resistant and (4) resistant groups The ratio between the diameter of the inhibition zone in milli *metres and the sensitivity to the anti* microbial agents is given in table 16

Two staphylococcal colonies from the same individual were assumed to be dif ferent strains if their antibiograms differed by 2 or more groups

4 Phage typing

The phages used were the basic set (21 phages) obtained from the Staphylococcus Reference Laboratory Colindale except phage 73 which was excluded at a meeting *of The International Committee on Phage* Typing of Staphylococci in Stockholm in 1959 In addition to these phages 81 (25) 82 (25) KS6 (139) 83A (136) and 83B (136) were used The series therefore in*cluded 25 phages which were divided* into the 5 groups listed in table 17 While most of the staphylococcal strains were lysed by more than one phage, several strains were lysed only by phage 42D or 187 They *were placed in two separate groups* IV and V Strains lysed by phages belonging to different groups have been placed in the miscellaneous group

The notations for recording the degrees of lysis given by the phages are shown in table 18 All lytic reactions from over 50 plaques to confluent lysis were regarded as strong reactions

Table 17 *Group distribution of phages*

Phage groups	Phages
I	29 52 52A, 79 80 81 82 KS6
II	3A, 3B, 3C, 55 71
III	6 7 42E, 47 53 54 75 77 83A 83B
IV	42D
V	187

Table 18. *Phage typing notations*

+ + +	Complete lysis"
+ +	"Strong lysis" More than 50 plaques.
+	Moderate lysis" 20—50 plaques.
±	"Weak lysis" Less than 20 plaques.

Strains which were not lysed by phages in the Routine Test Dilution (RTD) were retyped using phages 1 000 times more concentrated (RTD × 1 000) Non typable strains were recorded as NT (non typable)

IV Self-contamination and dispersal of Staph aureus by nasal carriers

A. Previous investigations.

The frequency of staphylococcal nasal carriers has been thoroughly examined and most investigations dealing with this problem have been reviewed by Lund (87) and Siboni (117) The carrier rate for adults lies between 30 and 50 per cent, higher nasal carrier rates being demonstrated in hospitals. The number of staphylococci in the nasal vestibule varies considerably from individual to individual but reports on the quantitative aspects of the problem are few (55 132, 133 142, 143 144)

Only few staphylococci are expelled directly into the air from the nose and mouth (31 54 87 117) It seems that dispersal of Staph. aureus by nasal carriers depends primarily upon the transfer of organisms from the site of multiplication in the nose to the skin, clothing and bedding by means of fingers and handkerchiefs (54 57) The pathway can be demonstrated by painting the anterior nares with a fluorescent substance (54)

Accordingly nasal carriers are frequently also staphylococcal skin carriers in contrast to individuals not carrying the organisms in the nose (39 54 87 89 97) and the nasal and skin strains are usually identical (55, 87 93 97 132 144 145)

Staphylococci have been isolated from the fingers (55, 68) and nail walls (67) of 70 to 90 per cent of nasal carriers less frequently from the face and back of the wrists (30, 55 97) and rather seldom and in smaller numbers from the axillae chest, abdomen, inguinal folds and legs (54 55 145)

In dispersal tests on 300 children Laurell and Wallmark (77) demonstrated that Staph. aureus could be isolated more frequently from the upper lip hands and clothes of individuals with large quantities of these organisms in nasal cultures than from those with small quantities. Similar results have been reported more recently (132 144) The number of staphylococci isolated from different skin areas of the same nasal carrier and from identical skin reas of different nasal carriers, varies considerably Little is known of the reason for this but reports on the quantitative aspects of the problems are few (55, 57)

Staphylococci are mainly liberated into the air by agitation of clothing and friction of the skin (31 54) Hare and Ridley (55) reported that nasal carriers who exercised in a cubicle wearing their ordinary every day clothing usually dispersed much larger numbers of staphylococci into the air than non-nasal carriers. However the ability of nasal carriers to disperse varied greatly and there was very little correlation between nasal counts and aerial dissemination. In 14 of 16 cases, identical strains were isolated from the nose and air samples Besides being a nasal carrier one of the two remaining individuals was

III Experimental design

The present investigation deals with examinations of staphylococcal carriers among the patients admitted to Medical Department B Haukeland Hospital Bergen The subjects were examined once daily usually for three consecutive days. Further details concerning the selection of patients and number of examinations are given in chapters IV V VI and VII

Two days before the first examination the patients were bathed or if bedridden washed on a stretcher They were given clean bed and washing equipment clean clothing and handkerchiefs Qualitatively and quantitatively all subjects received the same bed equipment (1 mattress 1 eiderdown 2 pillows and 2 sheets) and clothing (2 shirts, 2 pairs of trousers and a bathrobe)

To prevent contamination of the patients by staphylococci from their surroundings they were isolated from other staphylococcal carriers and the bed equipment and clothing were thoroughly examined for bacterial contamination before use.

The subjects were examined between 7.30 and 11 30 A M From the time they had gone to bed in the evening (8—9 P M) until they were examined the next morning they were not permitted to wash themselves or to leave their beds. When the examination was completed they were permitted to conduct themselves as usual in their rooms

Table 19. *Nasal count by age, sex and confinement to bed. 100 nasal carriers*

Nasal count	Mean age (46.1 years)	No. of patients				
		Sex distribution		Total	In bed	
		F (52)	M (48)		No. (58)	Per cent of total
$<10^4$	43.0	1	2	3	1	50
10^4-10^5	45.4	5	8	13	7	
10^5-10^6	46.3	16	18	34	20	59
10^6-10^7	46.1	27	16	43	24	56
$>10^7$	47.1	3	4	7	6	86

varied little and were 2.805 mill., 2.484 mill. and 2.686 mill. staphylococci respectively the mean count for all 300 examinations being 2.659 mill.

The material was divided into 5 groups on the basis of the mean nasal counts. Group 1 included counts less than 10^4 bacteria group 2 counts between 10^4 and 10^5 group 3 counts between 10^5 and 10^6 group 4 counts between 10^6 and 10^7 and group 5 counts larger than 10^7 bacteria. Table 19 gives the distribution of sex, average age and number of bedridden patients (out of bed for less than 2 hours daily) for each group. The majority of patients belonged to groups 3 and 4. Group 4 had an excess of women. The average age varied little from group to group, but the frequency of bedridden patients was greatest in the group with the highest nasal counts.

Nasal cultures yielding more than 10^7 staphylococci (group 5) were almost pure cultures, while those with less than 10^5 staphylococci (groups 1 and 2) always consisted of mixture with other organisms (Staphylococcus albus, Micrococcus catarrhalis and others) these usually constituting the greater part of the organisms. Groups 3 and 4 included the transition cases.

The individuals in group 5, who had the highest nasal counts, were more debilitated by their disease than the remaining patients. Two of the seven patients in this group died during their stay in hospital, one from uraemia and the other from haemolytic anaemia. Only two of the 93 patients in the other groups died while in hospital.

Antibiogram and phage patterns.

On the average antibiogram determinations and phage typing were performed on 7—8 colonies (ranging from 5 to 23) from the nasal cultures of each patient. In all, 112 strains were demonstrated Eighty nine patients had 1 strain, 10 patients had

Table 20. *Drug sensitivity of nasal strains in relation to nasal count.*

Nasal count	No. of strains		Resistant as per cent of total	
	Total	Resistant		
$<10^4$	3	0	0.0	5.9
10^4-10^5	14	1	7.1	
10^5-10^6	37	11	29.7	
10^6-10^7	50	20	40.0	44.8
$>10^7$	8	6	75.0	
Total	112	38	33.9	

a perineal carrier of a strain different from that isolated from his nose, and the perineal strain was dispersed

White (143) isolated staphylococci more frequently from the clothing of patients with high nasal counts than from those with low counts Later he reported that more than 20 staphylococcal colonies per c.ft. of air could be recovered from 35 per cent of air samples obtained after shaking the bed sheets of nasal carriers of large numbers of Staph. aureus but from only 8 per cent of air samples taken around non-carriers or carriers of smaller numbers (144)

Nasal carriers are frequently throat carriers (76 133 134) faecal carriers (14 17 66 91 92 138) and sometimes also perineal carriers (19) Dispersal of staphylococci most probably also takes place from these sites Sufficient attention has not been paid to this factor in earlier investigations of staphylococcal dispersal by nasal carriers

In the present study the difference in the ability of nasal carriers to disperse staphylococci was investigated Possible correlations between the numbers of staphylococci in the nose and on different skin sites and the dispersal into the air were studied

B Personal investigations

1 *Material and methods*

The material consisting of 52 women and 48 men between the ages of 14 and 75 years was selected in the following way All patients (2 014 in all) admitted to The Medical Department B from August 1962 to October 1963 were examined for staphylococci in the nose and throat and on the perineum. Thirty-six per cent of the patients were nasal carriers at the time of admission. From these patients 4—6 nasal cultures were obtained at two- to three day intervals On the last examination, the numbers of staphylococci in the axillae, vagina perineum and faeces were estimated

Patients who yielded staphylococci in the nose on all examinations were regarded as predominantly nasal carriers, provided they did not have staphylococcal lesions or too many organisms (1 000 or more Staph. aureus) in samples from the axillae, vagina perineum or faeces These criteria will be evident from the investigations reported in chapters VI and VII

One hundred and eleven patients fulfilled the criteria mentioned However 9 patients were unco-operative or too ill to be examined and in two patients the nasal staphylococci disappeared during the course of the subsequent investigations.

The remaining 100 patients were examined once daily for three consecutive days In order to keep experimental conditions approximately equal for all the patients they were bathed or washed on a stretcher and received clean clothing and bedclothes two days before the first examination

The methods of investigation are described in chapter II

2 *Results*

a. Nasal samples.

Quantitative estimations

The numbers of staphylococci in the nasal cultures of the 100 patients are given in the appendix table. The counts varied greatly from individual to individual, the lowest and highest counts being 960 and 32 08 mill. respectively However the variations on repeated examinations of the same individual were relatively moderate

The mean counts for the first second and third examinations of the 100 patients

Table 23. *Phage grouping of throat strains in relation to nasal count.* (*No. of strains*)

Nasal count	Typable (phage groups)						Non-typable	Total
	I	II	III	IV	V	Miscel-laneous		
$<10^4$	0	0	0	0	0	0	1	1
10^4-10^5	2	0	1	0	0	0	1	4
10^5-10^6	7	1	4	0	0	2	2	16
10^6-10^7	12	4	2	1	3	2	2	26
$>10^7$	1	1	2	0	1	0	1	6
Total	22	6	9	1	4	4	7	53

grouping of staphylococci in throat cultures in relation to nasal count. No definite correlation was observed. The majority of strains belonged to phage group I

Thirty-five patients had identical strains in the nose and throat whil 16 had different strains.

c. F cal mples

In selecting the material patients with staphylococci in the faeces were excluded from further investigation. Nevertheless, 6 of the 100 patients (Nos. 9 34 42, 79 84 and 97) yielded from 2 000 to 22,000 staphylococci per g of faeces Antibiogram determinations and phage typing were performed on 2 colonies from each sample. Four patients had identical strains in the nose and faeces, one patient yielded identical strains n the throat and faeces and one patient had a different strain in the faeces from that in the nose and throat.

d. Skin samples

Quantitative estimations.

The numbers of staphylococci demonstrated on the upper lip fingers and hands of the 100 nasal carriers are given in the appendix table. The highest counts for single bservations were 41,000 for the upper lip 457 000 for the fingers and 1.358 mill. for the hands.

Table 24 gives the frequency of positive skin samples in the 3 examinations. In each examination, the frequency was pproximately equal for identical skin areas and was highest for the fingers.

Table 24 *Frequency of positive skin samples from 100 nasal arriers.*

Area	Examination no.			At least one positive
	1	2	3	
Upper lip	70	73	74	89
Left fingers	83	81	87	96
Right fingers	54	56	47	77
Hands	77	76	78	90
Total area	90	91	90	96

Table 21 *Phage grouping of nasal strains in relation to nasal count. (No of strains)*

Nasal count	Typable (phage groups)							Non typable	Tot.
	I		II	III	IV	V	Miscellaneous		
	80/81	Others							
$< 10^4$	0	0	1	0	0	1	1	0	3
$10^4 - 10^5$	0	6	0	3	0	0	1	4	14
$10^5 - 10^6$	3	10	1	9	0	2	7	5	37
$10^6 - 10^7$	4	18	5	12	1	5	3	2	50
$> 10^7$	2	1	1	2	0	1	1	0	8
Total	9	35	8	26	1	9	13	11	112

2 strains and 1 patient had 3 different strains

Seventy four strains were sensitive to all antimicrobial agents used. Seventeen strains were resistant to penicillin alone. Twenty-one strains were resistant to sulphathiazole 20 to penicillin 13 to streptomycin 9 to tetracycline, 3 to erythromycin and 2 to chloramphenicol Table 20 gives the results of antibiogram determinations in relation to nasal count. The frequency of strains resistant to one or more antimicrobial agents increased with rising numbers of staphylococci in nasal cultures.

On phage typing 65 strains were lysed by RTD 36 strains by RTD × 1 000 and 11 strains were non typable Table 21 gives the results of phage typing in relation to staphylococcal nasal count. Patients with the highest nasal counts (groups 4 and 5) yielded less non typable but more 80/81 strains than those with the lowest counts (groups 1 2 and 3) The majority of strains belonged to phage groups I and III

b *Throat samples*

Staphylococci were also isolated from the throat of 51 of the 100 nasal carriers. The numbers of bacteria in throat cultures are given in the appendix table. The counts varied considerably from patient to patient, and also on repeated examinations of the same patient.

Antibiogram determinations and phage typing were performed on 1—2 colonies from each positive sample. In all 104 colonies were examined and 53 strains demonstrated Forty nine patients had 1 strain and 2 patients had 2 strains.

Eighteen strains were resistant to one or more antimicrobial agents. As seen in table 22 the frequency of resistant strains in throat samples was slightly greater for patients with the highest nasal counts (groups 4 and 5) than for those with the lowest counts (groups 1 2 and 3)

On phage typing 31 strains were lysed by RTD 15 by RTD × 1 000 and 7 strains were non typable. Table 23 gives the phage

Table 22 *Drug sensitivity of throat strains in relation to nasal count.*

Nasal count	No. of strains		Resistant as per cent of total
	Total	Resistant	
< 10	1	0	28.5
$10^4 - 10^5$	4	2	
$10^5 - 10^6$	16	4	
$10^6 - 10$	26	6	34 4
$> 10^7$	6	5	
Total	53	17	32 1

Table 27 *Correlation between staphylococcal counts from nose and fingers. Three examinations of 100 nasal carriers.*

Nasal count	No. of examinations	Fingers < 20 bact./sample No.	Per cent	≥ 1,000 bact./sample No.	Per cent
$<10^4$	13	10	77.0	0	0.0
10^4-10^5	34	14	41.2	1	2.9
10^5-10^6	117	8	6.8	22	18.8
10^6-10^7	115	3	2.6	82	71.3
$>10^7$	21	0	0.0	19	90.5
Total	300	35	11.7	124	41.3

Table 28. *Correlation between staphylococcal counts from nose and hands. Three examinations of 100 nasal carriers.*

Nasal count	No. of examinations	Hands < 500 bact./sample No.	Per cent	≥ 5,000 bact./sample No.	Per cent
$<10^4$	13	13	100.0	0	0.0
10^4-10^5	34	25	73.5	0	0.0
10^5-10^6	117	28	23.9	33	28.2
10^6-10^7	115	3	2.6	98	85.2
$>10^7$	21	0	0.0	21	100.0
Total	300	69	23.0	152	50.7

Table 29. *Correlation between staphylococcal nasal and skin counts. Correlation coefficients based on logarithms of observations (100 nasal carriers)*

Calculation based on	Method of calculation	N : U	N : F	N : H
3 replicate observations (at one-day intervals)	I	0.6907	0.7500	0.7659
	II	0.6455	0.7431	0.7761
Mean	I	0.7807	0.8502	0.8496
	II	0.7381	0.8218	0.8223

N = nose U = upper lip, F = fingers and H = hands.

Table 25 *Mean numbers of staphylococci isolated from various skin areas of 100 nasal carriers.*

Area	Method of cal culation	Examination no. 1	2	3	Mean
Upper lip	I	1 084	1,934	1,304	1 440
	II	1 089	1,939	1,308	1 445
Left fingers	I	6 505	9 199	6,225	7,310
	II	6,508	9,202	6,228	7,313
Right fingers	I	1,363	1 747	1 018	1,376
	II	1,371	1 755	1 027	1,384
Sum fingers	I	7 867	10,946	7,242	8 685
	II	7,879	10,958	7,254	8,697
Hands	I	40,225	36 640	46,715	41 193
	II	40,340	36,760	46,825	41,308

The frequency increased with the number of examinations but only to a negligible degree with the number of skin areas examined.

Table 25 gives the arithmetic mean counts for each skin area in the first, second and third examinations of the 100 patients and for the 300 individual observations. For counts below 20 for the fingers and upper lip and below 500 for the hands, the calculations are based on two alternative values 1 and 19 and 1 and 499 respectively In calculation method I the values are taken as 1 and in method II as 19 and 499 The difference in the results based on methods I and II was small. The greatest number of staphylococci was de monstrated on the hands and the smallest on the upper lip The mean counts for identical skin areas varied little on the 3

Table 26 *Correlation between staphylococcal counts from nose and upper lip Three examinations of 100 nasal carriers.*

Nasal count	No of examin ations	Upper lip < 20 bact./sampl No.	Per cent	≥ 1 000 bact./sample No.	Per cent
$<10^4$	13	12	92.3	0	0.0
10^4-10^5	34	30	88.2	0	0 0
10^5-10^6	117	31	26.5	13	11 1
10^6-10^7	115	8	7.0	37	32.2
$>10^7$	21	0	0 0	16	76.2
Total	300	81	27.0	66	22.0

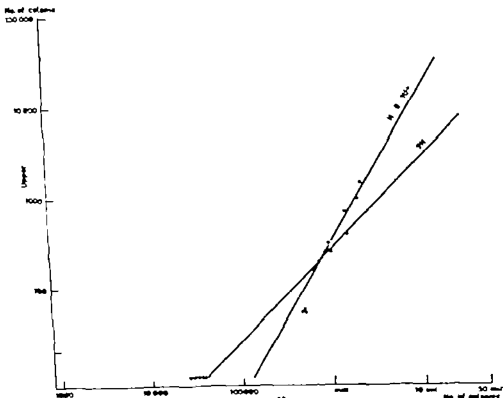


Fig. 5. Correlation between staphylococcal counts from nose and upper lip (mean counts 100 nasal carriers)

fingers, and figs. 5—7 of mean counts from nose and upper lip fingers and hands respectively The mean counts and re gression lines marked are calculated on the basis of method I Values for single counts below 20 and mean counts below 10 are plotted on the bcissa. Within wide limits, the numbers of staphylococci in skin samples increased with rising nasal counts. It is evident from figs. 4 and 6 that the mean counts were much less scattered than the single counts.

Abdominal skin samples were also obtained from the first 50 patients. Three patients each had 1 positive sample which yielded 40 120 and 40 staphylococci respectively

In selecting the material, patients with 1,000 or more staphylococci in the axillae and perineum were excluded from further examinations. Nevertheless, two patients (Nos. 12 and 25) yielded from 2,000 to 6 000 staphylococci in samples from the axillae on the second and third examina tions, and 3 patients (Nos. 9 28 and 69) from 1,000 to 4,000 staphylococci from the perineum in one or two of the three examinations.

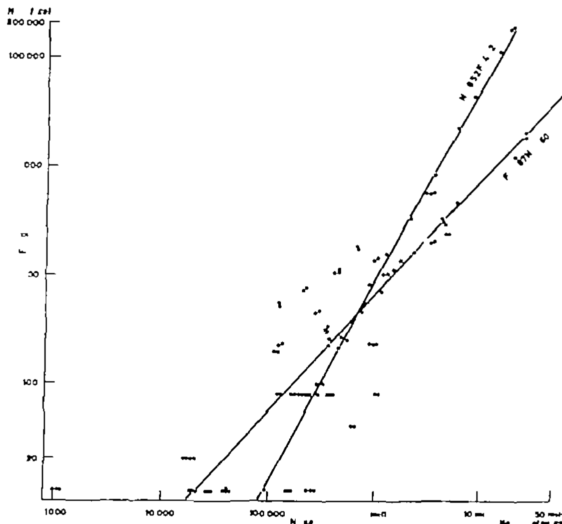


Fig 4 Correlation between staphylococcal counts from nose and fingers (single counts, 100 nasal carriers)

examinations. More staphylococci were isolated from the fingers of the left hand than from the right.

Tables 26—28 show the correlation between the nasal counts and the counts from upper lip fingers and hands respectively on 3 examinations of the nasal carriers. Within wide limits, the skin counts increased with rising nasal counts.

The frequency distribution of the skin and nasal counts was found to approximate the log normal form. For statistical analysis, the counts were therefore transformed to a logarithmic scale and all computations made on the transformed figures For counts below 20 for the fingers and upper lip and below 500 for the hands, the calculations were based on two alternative values as mentioned above.

Table 29 gives the correlation coefficients between logarithms of nasal and skin counts. The correlations were good being best between the nose and hands and least good between the nose and upper lip.

Fig 4 illustrates the correlation between logarithms of single counts from nose and

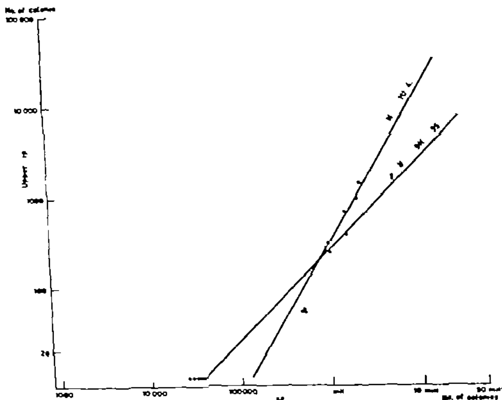


Fig. 5 Correlation between staphylococcal counts from nose and upper lip (mean counts, 100 nasal carriers)

fingers, and figs. 5—7 of mean counts from nose and upper lip, fingers and hands respectively. The mean counts and regression lines marked are calculated on the basis of method I. Values for single counts below 20 and mean counts below 10 are plotted on the abscissa. Within wide limits, the numbers of staphylococci in skin samples increased with rising nasal counts. It is evident from figs. 4 and 6 that the mean counts were much less scattered than the single counts.

Abdominal skin samples were also obtained from the first 50 patients. Three patients each had 1 positive sample which yielded 40, 120 and 40 staphylococci respectively.

In selecting the material, patients with 1,000 or more staphylococci in the axillae and perineum were excluded from further examinations. Nevertheless, two patients (Nos. 12 and 25) yielded from 2,000 to 6,000 staphylococci in samples from the axillae on the second and third examinations, and 3 patients (Nos. 9, 28 and 69) from 1,000 to 4,000 staphylococci from the perineum in one or two of the three examinations.

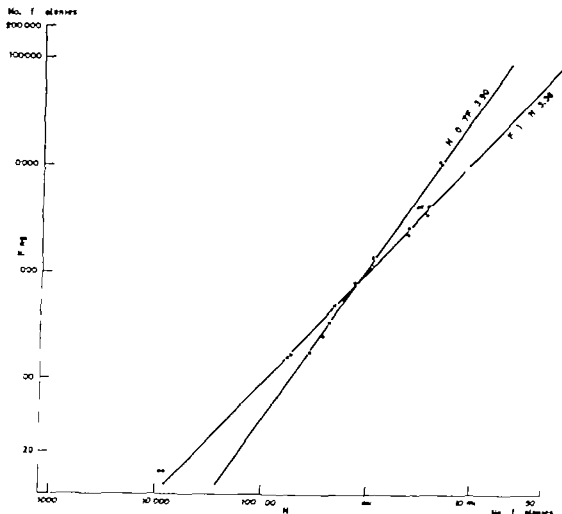


Fig 6. Correlation between staphylococcal counts from nose and fingers (mean counts, 100 nasal carriers)

Antibiogram and phage patterns

Antibiogram determinations and phage typing were performed on 278 colonies from the upper lip 587 colonies from the fingers and 456 colonies from the hands. *Altogether 1 321 colonies* were examined The reactions of 1,311 colonies to the anti microbial agent and phages were either identical with those of the respective nasal strains or showed such slight variations that the strains were not assumed to be different. The remaining 10 colonies gave sensitivity patterns identical with the nasal strains but phage typing results were in decisive. Serological typing revealed that these strains were most probably identical

In the 7 positive samples from the axillae and abdomen the reactions of 8 colonies to antimicrobial agents and phages were examined. The reactions were identical with the respective nasal strains.

Antibiogram determinations and phage typing were performed on one colony from each of the positive perineal samples One patient yielded identical strains on the perineum and in the nose a second patient had identical strains on the per

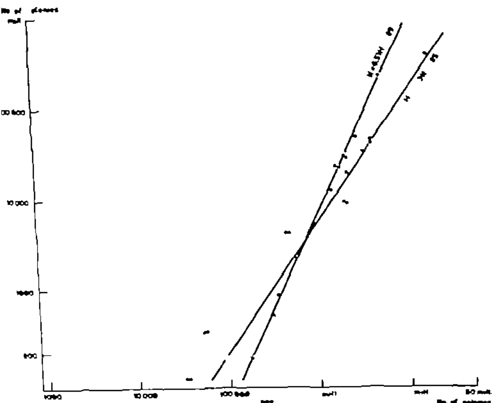


Fig. 7 Correlation between staphylococcal counts from nose and hands (mean counts, 100 nasal carriers)

neum and in the throat and third had perineal strain different from that in the nose and throat.

c. Staphylococcal aerial dissemination on bed making

Quantitative estimations

The appendix table shows, for the 100 nasal carriers, the degree of staphylococcal air contamination on bed making. When staphylococci were not demonstrated in the dispersal experiments (counts less than 25) the calculation of the mean counts for the 3 examinations was based on two alternative values, 1 (method I) and 24 (method II) respectively as mentioned in chapter II. Dispersal of staphylococci was demonstrated from 93 patients on the first examination and from 91 patients on the other examinations. The air counts varied considerably from patient to patient — from less than 25 to 43 400 Staph. aureus particles — but the day to day variations for the same patient were moderate.

The mean counts for the first second and third examinations of the 100 patients were 2,463 (2 465) 2 446 (2 448) and 2,086 (2,088) staphylococcal particles respectively and for the 300 examinations

Table 30 *Correlation between staphylococcal nasal counts and degree of aerial dissemination on bed making Three examinations of 100 nasal carriers*

Nasal count	No. of examinations	Degree of air contamination			
		< 100 bact.*/sample		≥ 1 000 bact. /sample	
		No.	Per cent	No.	Per cent
$<10^4$	13	13	100.0	0	0.0
10^4-10^5	34	18	52.9	0	0.0
10^5-10^6	117	12	9.6	26	22.2
10^6-10^7	115	1	0.9	82	71.3
$>10^7$	21	0	0.0	19	90.5
Total	300	44	14 7	127	42.3

* Staph. aureus particles.

Table 31 *Correlation of staphylococcal nasal and skin counts to aerial dissemination on bed making Correlation coefficients based on logarithms of observations (100 nasal carriers)*

Correlation based on	Method of calculation	N.A	U.A	F.A	H.A	(H+F).A
3 replicate observations (at one-day intervals)	I	0.8043	0.6745	0.8199	0 7954	0.8523
	II	0 7988	0 6573	0.8340	0.8122	0.8395
Mean	I	0.8611	0 7869	0.9203	0.8986	0.9366
	II	0.8304	0 7637	0.9050	0.8797	0.9011

N = nose, U = upper lip, F = fingers, H = hands and A = air contamination

Table 32 *Multiple correlation of combinations of mean staphylococcal nasal and skin counts to air counts.*
Multiple correlation coefficients based on logarithms of observations (100 nasal carriers)

Method of calculation	$R_{A(F+H.N)}$	$R_{A(N.U)}$	$R_{A(F+H.U)}$	$R_{A(F+H.N.U)}$
I	0.9387	0.8804	0.9378	0.9394
II	0.9120	0.8599	0.9076	0.9149

N = nose, U = upper lip, F = fingers, H = hands and A = air contamination

$R_{y(x_1, \ldots x_n)}$ is the multiple correlation coefficient between the dependent variable y and the independent variables $x_1 \ldots x_n$.

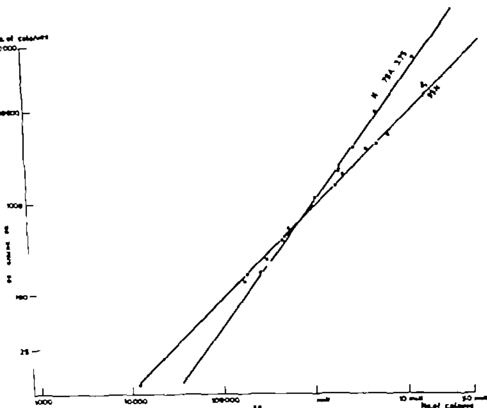


Fig. 8. Correlation between staphylococcal counts from nose and air (mean counts, 100 nasal carriers)

2,331 (2,333) particles. The figures in parenthesis are calculated on the basis of method II. The difference in the results derived from the two methods of calculation was small and the mean counts were approximately equal for the 3 examinations.

Table 30 gives the correlation between nasal and air counts on 3 examinations of the nasal carriers. Within wide limits, the air counts increased with rising numbers in nasal cultures.

The frequency distribution of the counts was found to approximate the log-normal form. For statistical analysis, the counts were therefore transformed to a logarithmic scale and all computations made on the transformed figures.

Table 31 gives the correlation coefficients of logarithms of nasal and skin counts to air counts. The difference in the results based on the two methods of calculation was small. The correlation was least good between upper lip and air contamination, and best between fingers plus hands and air contamination.

Figs. 8—10 illustrate the correlation between mean counts from nose, fingers and fingers plus hands and mean counts of air contamination. The points marked and

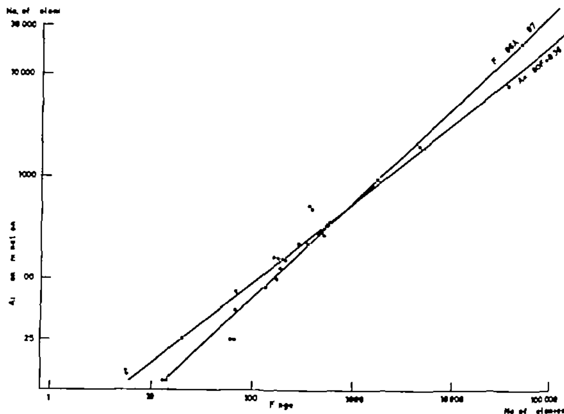


Fig 9 Correlation between staphylococcal counts from fingers and air (mean counts, 100 nasal carriers)

the regression lines represent calculation method I Mean counts below the lowest values on the abcissa and ordinate are plotted on the co-ordinates It is evident from the figures that the correlation between fingers and hands combined and air contamination was better than between nose and air contamination

Table 32 gives the multiple correlation coefficients of combinations of mean counts from nose, upper lip and fingers plus hands to mean air counts. The correlation achieved when all the combinations of measured factors which might conceivably influence air contamination were taken into consideration was only slightly better than that formerly demonstrated (table 31) between fingers plus hands and air contamination. Quantitative estimation of staphylococci in the nose and on the upper lip after measurement of the numbers on the fingers and hands thus provided only slight additional information concerning the air contamination.

Antibiogram and phage patterns

Antibiogram determinations and phage typing were performed on 1 413 colonies from air samples The reactions of 1 379 colonies to the antimicrobial agents and phages were either identical with those of the respective nasal strains, or showed such slight variations that the strains were not assumed to be different. Nine of the remaining 34 strains gave resistance patterns identical with the nasal strains but phage typing results were indecisive. Serological

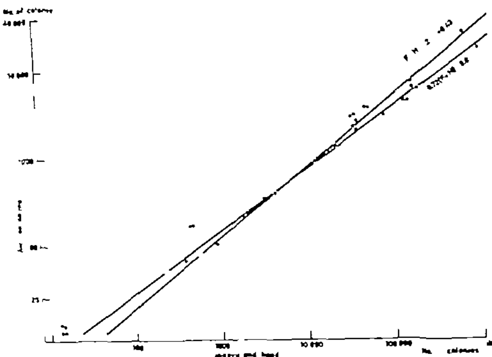


Fig. 10. Correlation between staphylococcal counts from fingers + hands and air (mean counts, 100 nasal carriers)

typing showed that in 6 of the 9 cases the air and nasal strains were identical. Consequently 1,385 of 1 413 colonies from air samples were most probably identical with the nasal strains and 28 colonies were different.

Twenty-six of these 28 colonies were demonstrated in samples from which all staphylococcal colonies were examined that is, in 161 samples yielding 462 colonies. The remaining 2 colonies were demonstrated in the other 139 samples in which only 951 of 6,576 staphylococcal colonies were examined. These 28 colonies were not included in the calculation of the air contamination as they were thought to be due to contamination from the environment.

5 *Discussion.*

In the present investigation an important problem was whether the staphylococci demonstrated on the various skin areas and in the air samples were really due to dispersal from the nose — directly or indirectly — and not to contamination from the surroundings or dispersal from other sites of multiplication such as the throat, vagina, perineum or faeces. As virtually all staphylococcal colonies examined in the skin and air samples were identical with the nasal strains, contamination from the surroundings must have been negligible. It is also evident that dispersal from the throat was of minor importance since 16 of the 100 patients had different nasal and

throat strains and the latter were not demonstrated in the skin and air samples. In previous investigations bacterial dissemination by throat carriers has also been assumed to be unimportant (51 52 53 54 111 137) Only a few patients had positive samples from the axillae, perineum and faeces and none from the vagina (cf. selection of the material) The positive samples yielded such small counts that dispersal of staphylococci from these sites was most probably negligible (cf. chapters VI and VII) Moreover the perineal and faecal strains in four of these patients were different from the nasal strains, and the former were not demonstrated on other skin areas nor in air samples. Other breeding grounds for staphylococci apart from the nasal vestibule and the sites mentioned here, are seldom demonstrated.

The foregoing statements indicate that the majority of staphylococci on the upper lip fingers and hands are due to contamination from the nose. This is also supported by the good correlation between the nasal and skin counts In addition very few staphylococci were demonstrated on skin areas (e g skin of the abdomen) which did not come into direct contact with the nasal vestibule.

The difference in the ability of nasal carriers to disperse staphylococci into the air must largely depend on the number of these organisms in the nose and on the skin The linear correlation was better between the counts from fingers plus hands and air contamination than between the nasal and air counts. Multiple correlation analysis revealed that insignificant additional information on the ability to disperse the organisms into the air was attained by estimating the number of bacteria in the nose and on the upper lip when the counts from the fingers and hands were known Consequently estimation of the number of organisms on the fingers and hands will give the best basis for determining whether a nasal carrier is a 'heavy disperser' or not. Hare and Ridley (55) also observed correlation between the numbers of staphylococci dispersed and the extent of surface contamination. The results of the present investigation support the theory of Hare (57) concerning the mode of infection the bed clothes are infected from the skin, particularly from the fingers and hands, which in their turn are infected from the vestibule of the nose.

As shown in the present study the numbers of staphylococci on the skin and their dispersal into the air increased with rising nasal counts In investigations where other quantitative methods have been used similar tendencies have been reported for nasal carriers of both staphylococci (77 132 143 144) and streptococci (50) However Hare and Ridley (55) observed very little or no correlation between the number of organisms in the nose and the intensity and extent of skin contamination and ability to disperse This may be due partly to their technique which permitted only a rough estimation of the number of staphylococci in the nose and on the skin and partly to the difficulty of maintaining a constant technique in the dispersal experiments In the majority of studies on nasal carriers, insufficient attention has also been paid to the dispersal of staphylococci from sites of multiplication outside the nasal vestibule (faeces perineum etc)

On the other hand it must be emphasized that in the present work the patients were submitted to approximately equal experimental conditions. All had bathed and received similar clean clothing and bedclothes 2 days before the first examination. They were all supplied with handkerchiefs. None were permitted to wash themselves during the last 12—14 hours before the examinations, or to get out of bed during this time. In addition no patients

had such complaints as those described for "cloud babies" (52). All this has probably contributed to the good correlation demonstrated between the nasal, skin and air counts.

Identical strains were nearly always demonstrated in the nasal, skin and air samples. Similar findings have been made in other investigations (55, 87, 97, 132, 144, 145) but the results have not been quite so good. As shown in the present and a previous investigation (109) two staphylococcal nasal strains can be isolated from about 10 per cent of nasal carriers. If only one colony from each nasal and skin sample is examined, different strains will be found fairly often. In the present study this source of error was avoided by examining several colonies from each sample. In previous investigations less attention has also been paid to the dispersal of staphylococci from sites of multiplication outside the nasal vestibule, where other strains may appear (perineum, faeces, vagina, axillae). In addition, the individuals in the present study were isolated from other staphylococcal carriers in order to prevent contamination from their surroundings.

4. *Summary and conclusions*

1. From the patients admitted to a medical department during the course of 14 months, 100 persistent nasal carriers of Staph. aureus were selected.

In these patients the numbers of staphylococci in the nasal vestibule and on various skin sites, and the ability to disperse the organisms into the air on bed making were examined.

2. The numbers of staphylococci in the nasal cultures varied greatly from individual to individual — from less than 1,000 to several millions — but the day to day variations for the same individual were relatively small.

Nasal samples yielding more than 10^7 Staph. aureus were practically pure cultures while samples with lower counts generally yielded other organisms as well. Debilitated patients had the highest nasal counts.

Altogether 112 staphylococcal nasal strains were isolated. The frequency of antibiotic resistant strains increased with rising nasal counts.

3. The numbers of staphylococci on the upper lip, fingers and hands, and the frequency of positive samples from these sites increased within wide limits with rising nasal counts. Staphylococci were seldom demonstrated on the skin of the abdomen, and then only in insignificant numbers.

In every case the nasal and skin strains were identical.

These investigations support the view that the majority of staphylococci on the skin of nasal carriers are due to contamination from the nasal vestibule.

4. Within wide limits, the dispersal of staphylococci into the air on bed making increased with rising nasal counts but there was better correlation between the number of staphylococci on the skin (fingers and hands) and aerial dissemination than between nasal and air counts.

Ninety-eight per cent of the staphylococcal colonies examined in air samples were identical with the nasal and skin strains.

The heaviest dispersers of staphylococci among nasal carriers are those individuals who yield the highest numbers of these organisms on the skin (fingers and hands). These subjects usually also have the highest numbers in nasal cultures.

5. Fifty-one of the 100 patients yielded staphylococci in throat samples and 16 had different strains in the nose and throat. In these patients the throat strains were not demonstrated in skin and air samples. Presumably throat carriers are of minor importance, compared with nasal carriers in the dispersal of Staph. aureus.

V Staphylococcal nasal carriers treated with framycetin-gramicidin nasal spray

A Previous investigations

Nasal carriers play a considerable part in the development of staphylococcal infections Danbolt (28) demonstrated this in the case of furunculosis as early as 1931 Later this was shown to apply both to furunculosis (126 130) and to other staphylococcal skin lesions (27 35 65 108 126) Perinatal pyodermias and puerperal abscesses may also be due to nosocomial infections (78 88 120) and post operative septic lesions are often thought to result from autoinfection with staphylococci from the nose (23 95 140 148)

Local treatment of the nasal mucosa with antibiotics can reduce the frequency of recurrent staphylococcal skin lesions among nasal carriers (27 46 65 127) This treatment also seems to reduce the frequency of staphylococcal infections in maternity wards (4 40 72 75 121) In surgical wards however the results are not quite so uniform and encouraging (42 43 63 110 121 140) In several investigations, other measures have been taken concurrently with antibiotic therapy and the individual effect has been difficult to assess.

Various antibiotics have been used in the treatment of staphylococcal nasal carriers and a definite reduction in nasal carriage has usually been demonstrated during and just after treatment (33 44 45, 129 141) But in some investigations less favourable results have been obtained (90 102)

In recent years, framycetin has been used alone or in combination with gramicidin in order to reduce the frequency of nasal carriers In several investigations the results have been good (8 71 115 123) but not in all (102)

The efficiency of intranasal application of antibiotics in reducing skin contamination and aerial dissemination of staphylococci by nasal carriers has seldom been studied (98 132) and little is known of the quantitative side of the problem. The results of investigations of this aspect of the problem will be reported here.

B Personal investigations.

1 Material and methods

Nasal samples from the nurses and doctors in the department were obtained every other week for 1½ years. Thirty individuals who had been carriers of the same staphylococcal strain for 2—6 months were selected and the incidence and quantity of these organisms in the vestibule of the nose were determined once daily for 3 days before treatment with framycetin gramicidin nasal spray Nasal cultures were obtained the day after completing therapy and again at 1 week intervals.

Forty of the 100 nasal carriers in chap-

ter IV were also treated with framycetin-gramicidin nasal spray. The numbers of staphylococci on the skin and the dispersal into the air on bed making were examined once daily for 3 days before treatment (the results are reported fully in chapter IV) and on the day after completing therapy.

Twenty of the 40 patients remained in the department for at least a further 10 days, the incidence and quantity of staphylococci in the nasal vestibule being determined on the 4th and 10th days after completing treatment. In 3 of these patients, skin and air samples were obtained several times after treatment.

In order to keep experimental conditions approximately equal before and after treatment, the 40 patients were bathed or washed on a stretcher and received clean clothes and bedclothes 2 days before the first pre-treatment examination and 2 days before the post-treatment examination.

The framycetin-gramicidin nasal spray was used 4 times daily. The spray fluid was an isotonic solution containing 1.25 per cent framycetin, 0.005 per cent gramicidin, 0.25 per cent methoxedrin and 0.002 per cent phenylmercurinitrate. The patients were treated for 3 days and the personnel for 7 days.

The methods of investigation are described in chapter II. In assessing the frequency of nasal carriers, nasal cultures which did not yield staphylococci in the first dilution (1:40) were regarded as negative.

2. *Results*

Tables 33 and 34 give the frequency of nasal carriers of Staph. aureus among the personnel and patients after treatment. The frequency was lowest for the personnel who had been treated longest, and in both groups it was lowest on the day after completion of treatment and rose rapidly in the course of the following 1—2 weeks. Antibiogram determinations and phage typing were performed on 1—2 colonies from all positive nasal cultures. Only 3 individuals in each group yielded different strains after therapy.

Figs. 11 and 12 illustrate the mean nasal counts from the personnel and patients before and after treatment. In both groups there was a marked reduction from high pre-treatment values to less than 0.01 per cent of the original numbers the day after completion of therapy. The nasal counts from the majority of individuals in both

The preparation was supplied by Nyco, Oslo.

Table 33. *Staphylococcal nasal carriage after treatment with framycetin-gramicidin nasal spray for seven days.*
(*30 members of the personnel*)

Day after treatment	No. of samples		Per cent of samples positive
	Positive	Negative	
1	3	27	10
7	17	13	57
14	23	7	77
21	25	5	81
28	27	3	90

Table 34. *Staphylococcal nasal carriage after treatment with framycetin-gramicidin nasal spray for three days.*
(*20 patients*)

Day after treatment	No. of samples		Per cent of samples positive
	Positive	Negative	
1	8	12	40
4	11	9	55
10	18	2	90

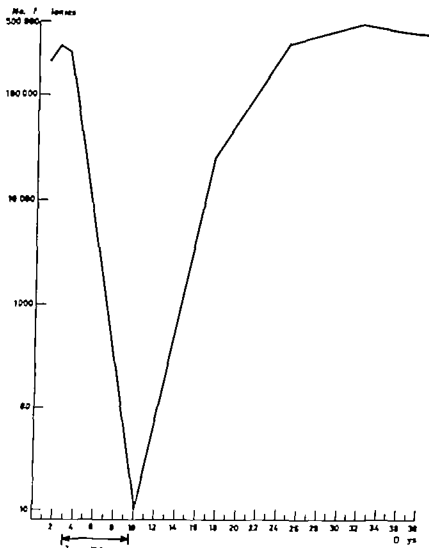


Fig 11 Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 30 nasal carriers)

groups were still low 4 and 7 days later but during the subsequent week the mean counts for both groups rose to about the same level as before treatment.

Quantitative nasal cultures were obtained for 10 days from 10 untreated subjects (patients and personnel) who had been shown by weekly examinations (covering 1 to 2 months) to be persistent staphylococcal nasal carriers. Fig 13 illustrates the mean numbers of *Staph. aureus* in daily samples from these individuals Only minor variations were observed

Table 35 gives the frequency of staphylococcal positive nasal throat, skin and air samples from 40 nasal carriers before and after treatment From the throat staphylococci were isolated almost as frequently after treatment as before but in the other samples the organisms were seldom demonstrated after treatment.

Table 36 and figs. 14 and 15 give the mean counts in nasal, skin and air samples

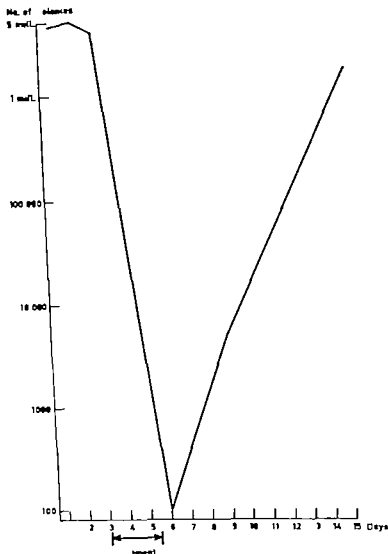


Fig. 12. Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 20 nasal carriers)

from 40 nasal carriers before and after treatment. For all samples, a marked reduction was demonstrated from high values before treatment to extremely low values the day after treatment was completed.

Three patients had positive perineal samples (from 2,000 to 8 000 staphylococci) the day after completing treatment.

Sixteen of the 40 patients yielded positive post-treatment nasal cultures. Antibiogram determinations and phage typing were performed on 18 colonies from these

Table 35 *Frequency of staphylococcal positive samples before and after treatment with framycetin-gramicidin nasal spray for three days*
(40 nasal carriers)

Day of treatment	Nose		Throat		Upper lip		Fingers		Hands		Air contam.	
	No	Per cent	No	Per cent	No.	Per cent	No.	Per cent	No	Per cent	No.	Per cent
−3	40	100 0	13	32.5	36	90 0	40	100 0	39	97.5	40	100.0
−2	40	100 0	12	30 0	35	87.5	40	100 0	39	97.5	40	100.0
−1	40	100 0	11	27.5	37	92.5	40	100.0	39	97.5	40	100.0
+1	16	40 0	8	20 0	8	20.0	5	12.5	1	2.5	9	22.5

− = before treatment, + = after treatment.

Table 36. *Mean staphylococcal nasal, skin and air counts before and after treatment with framycetin-gramicidin nasal spray for three days.*
(40 nasal carriers mean counts in thousands)

Day of treatment	No. of bacteria				
	Nose	Upper lip	Fingers	Hands	Air contam.
−3	4 614 650	2.380	13.925	64 163	4.280
−2	3 746.500	3 182	13 986	51.263	4 433
−1	4 600 080	2.302	17.291	57 100	3.888
+1	0 155	0.009	0.003	0 013	0.012

Calculation method I − = before treatment,
+ = after treatment.

samples. In all cases identical strains were demonstrated before and after treatment. Altogether 25 staphylococcal colonies were demonstrated in the 14 positive samples from upper lip fingers and hands of the 40 patients after treatment. Antibiogram determinations and phage typing were performed on 15 colonies Only 1 colony differed from the corresponding nasal strain Seventeen Staph. aureus colonies were demonstrated in the air samples after treatment. Seven colonies were different from the respective nasal strains Skin and air sample colonies which differed from the respective nasal strains were assumed to be due to environmental contamination and were not included in the calculations of skin and air contamination.

Eight patients had positive throat samples after treatment. Antibiogram determinations and phage typing were performed on 10 colonies from these samples. Five patients had identical throat strains before and after therapy Staphylococci were not demonstrated in skin and air samples from 3 patients who yielded positive throat cultures and negative nasal cultures after treatment.

Three patients were examined several times after completing treatment with nasal spray Table 37 shows the results of these post treatment examinations which

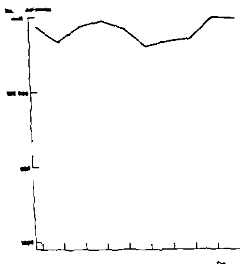


Fig. 13 Mean staphylococcal nasal counts from 10 untreated nasal carriers.

demonstrated that the number of organisms on the skin, and the dispersal into the air increased with increasing nasal counts.

Antibiogram determinations and phage typing were performed on a total of 68 Staph. aureus colonies from the samples of these 3 patients. Apart from 2 colonies in the air samples, all strains from each patient were identical.

One patient had mild side-effects from the treatment in the form of nasal stenosis and mild dyspnoea. The symptoms disappeared as soon as treatment was discontinued.

Staphylococcal strains resistant to framycetin and gramicidin were not observed during the investigation.

3 *Discussion.*

Nasal spray therapy has only a temporary influence on the carrier status. Staphylococci are often demonstrated after the completion of treatment the interval before the nasal samples become positive varying considerably (71 102 115 123) This may partly be due to differences in technique (71 102) and in the staphylococcal nasal counts before treatment. It has in fact been shown that patients who were recolonized after nasal application of antibiotics, yielded higher pre-treatment nasal counts than patients who became non-carriers (152) The individuals in the present investigation were accounted persistent carriers — carriers with high nasal counts (117) — while in other investigations (71 123) there were probably more occasional carriers — individuals yielding few nasal staphylococci. The results are probably also dependent on the duration of treatment and the care with which it is carried out (115) In the present investigation, checks were made once or twice daily to see that the spray bottle was used correctly

In the majority of cases, the demonstration of staphylococci after treatment was probably due only to persistence of the original strains in the vestibule of the nose. Stratford et al. (123) surmised that the recolonization was due to exogenous staphylococci but as phage typing was not performed, they were unable to prove this. In the majority of patients in the present study the pre and post-treatment nasal strains were identical. There were very few or no staphylococci on the skin and bedclothes after treatment so that dispersal to the nose from these sites was most unlikely Re-infection from the throat was, on the other hand possible in some cases. In the majority however persistence of the original organisms in the vestibule of the nose was the most reasonable explanation. This is in accordance with the results of other investigations (71 102)

One of the 3 individuals in the personnel group who changed strains had negative nasal cultures for 22 days, but the day after

Table 35 *Frequency of staphylococcal positive samples before and after treatment with framycetin-gramicidin nasal spray for three days*
(40 nasal carriers)

Day of treatment	Nose		Throat		Upper lip		Fingers		Hands		Air contam.	
	No.	Per cent	No	Per cent	No.	Per cent	No	Per cent	No.	Per cent	No.	Per cent
−3	40	100 0	13	32.5	36	90.0	40	100 0	39	97.5	40	100.0
−2	40	100 0	12	30 0	35	87.5	40	100 0	39	97.5	40	100 0
−1	40	100.0	11	27.5	37	92.5	40	100.0	39	97.5	40	100.0
+1	16	40 0	*8*	*20 0*	*8*	*20.0*	*5*	*12.5*	*1*	*2.5*	*9*	*22.5*

− = before treatment, + = after treatment.

Table 36 *Mean staphylococcal nasal, skin and air counts before and after treatment with framycetin-gramicidin nasal spray for three days*
(40 nasal carriers, mean counts in thousands)

Day of treatment	No. of bacteria*				
	Nose	Upper lip	Fingers	Hands	Air contam.
−3	4 614 650	2.380	13.925	64 163	4.280
−2	3 746.500	3.182	13.986	51.263	4 433
−1	4 600 080	2.302	17.291	57 100	3 888
+1	*0 135*	*0.009*	*0 003*	*0.015*	*0.012*

Calculation method I − = before treatment
+ = after treatment.

samples In all cases, identical strains were demonstrated before and after treatment. Altogether 25 staphylococcal colonies were demonstrated in the 14 positive samples from upper lip fingers and hands of the 40 patients after treatment Antibiogram determinations and phage typing were performed on 15 colonies. Only 1 colony differed from the corresponding nasal strain Seventeen Staph. aureus colonies were demonstrated in the air samples after treatment. Seven colonies were different from the respective nasal strains. Skin and air sample colonies which differed from the respective nasal strains were assumed to be due to environmental contamination and were not included in the calculations of skin and air contamination

Eight patients had positive throat samples after treatment. Antibiogram determinations and phage typing were performed on 10 colonies from these samples. Five patients had identical throat strains before and after therapy Staphylococci were not demonstrated in skin and air samples from 3 patients who yielded positive throat cultures and negative nasal cultures after treatment

Three patients were examined several times after completing treatment with nasal spray Table 37 shows the results of these post treatment examinations, which

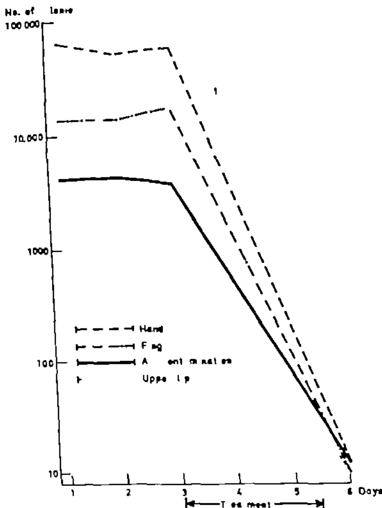


Fig 15. Staphylococcal skin and air counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 40 nasal carriers)

with those organisms which ppeared in greatest numbers. These observations are few in umber but there is probably quantitative factor in the transfer of staphylococci from one individual to another

Although staphylococci can be demonstrated in nasal cultures after treatment, a purely qualitative assessment does not give a true picture of the effect of treat ment. Porter et al. (102) did not undertake quantitative investigations but they were nevertheless aware of this Although the use of framycetin seemed to have little effect on the over-all pattern of staphylococcal nasal carriage it may be that

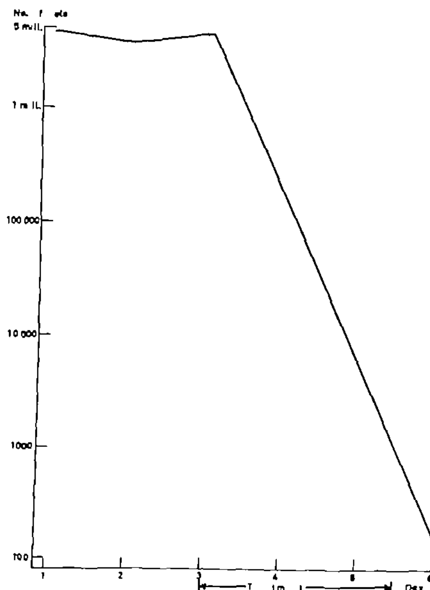


Fig. 14. Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 40 nasal carriers)

she began nursing a patient isolated for a severe staphylococcal pyodermia (patient 1 chapter VII) her nasal cultures yielded a strain identical with that harboured by the patient.

In one of the 3 patients who changed strains the one developed in the nose after treatment was identical with the strain yielded by the perineum. Another of these 3 patients, who was placed in the same room as a perineal carrier who dispersed large numbers of staphylococci yielded after 3 days positive nasal cultures of a strain identical with that of the perineal carrier

Routine examination of air contamination in the wards and charting of all staphylococcal carriers among personnel and patients showed that those individuals who changed their strains were recolonized

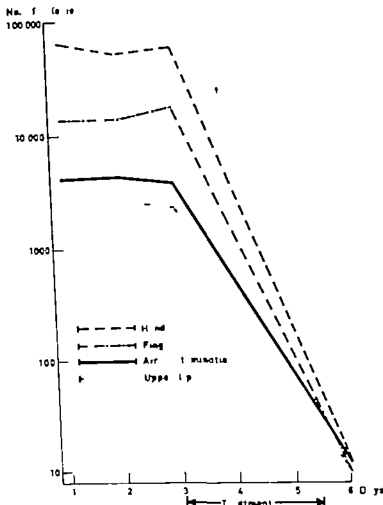


Fig 15. Staphylococcal skin and air counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 40 nasal carriers)

with those organisms which appeared in greatest numbers. These observations are few in number but there is probably a quantitative factor in the transfer of staphylococci from one individual to another

Although staphylococci can be demonstrated in nasal cultures after treatment, a purely qualitative assessment does not give a true picture of the effect of treatment Porter et al. (102) did not undertake quantitative investigations but they were nevertheless aware of this "Although the use of framycetin seemed to have little effect on the over-all pattern of staphylococcal nasal carriage it may be that

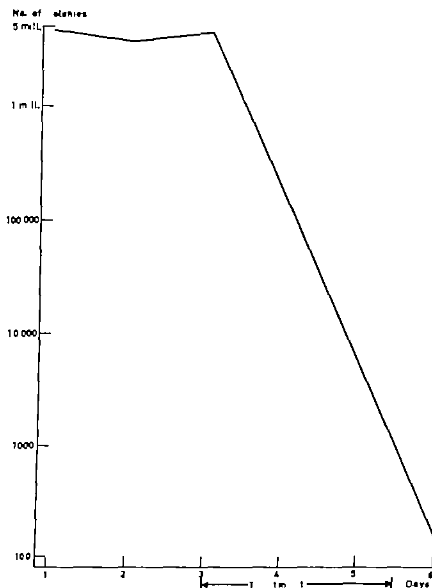


Fig 14 Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 40 nasal carriers)

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Routine examination of air contamination in the wards and charting of all staphylococcal carriers among personnel and patients showed that those individuals who changed their strains, were recolonized

Twenty-seven (90 per cent) individuals in the personnel group and 1? (60 per cent) in the patient group had negative nasal cul tures on the day follow ng completion of treatment. However the frequency of positive cultures increased rapidly in both groups in the follow ng 1—2 weeks.

For both groups, the staphylococcal na sal counts fell from high pre-treatment values to less than 0 01 per cent of the origi nal counts on the day after completion of treatment. They remained relatively low for 4—7 days, then rose during the following week to about the same level as before treatment.

Eighty to ninety per cent of the individuals with positive nasal cultures after treatment yielded the same strain before and after therapy It is assumed that in the majority of cases the original organisms persisted in the nose.

2. Forty patients who were nasal car riers of large n mbers of staphylococci were treated with framycetin-gramicidin nasal spray for 3 days.

The quantity of staphylococci in the nose on the upper lip fingers and hands fell from high pre-treatment values to very low values on the day after completion of therapy The same applied to aerial dissemination of staphylococci on bed mak ing When the staphylococcal nasal counts began to rise after treatment the skin and air contamination also rose.

Treatment by framycetin-gramicidin nasal pray is regarded as a valuable measure for preventing the dissemination of staphylococci from nasal carriers.

topical antibiotics of this type reduce the density of nasal organisms and so decrease the environmental contamination from this source.

In the present study a marked reduction was demonstrated in the staphylococcal skin and air counts from high values before treatment to almost negligible numbers after its completion. If there is a quantitative factor in the development of staphylococcal lesions a simple treatment such as antibiotic nasal spray should therefore contribute to reducing the frequency of infections with these organisms. As mentioned previously this has been shown in some investigations but not in all (63 121).

The staphylococcal skin and air counts fell during treatment with nasal spray approximately in parallel with the number of organisms in the nasal samples. This might be due to accidental contact of skin and spray e.g. when the cap was screwed on and off or via handkerchiefs but the main reason was probably that the number of dispersable organisms in the nasal vestibule fell to a minimum.

Only one of the individuals in the present study had side-effects from the therapy and these were very mild. In large doses, gramicidin may produce lung infiltrations in rabbits (105). Rubbo (113) and Gremeaux (48) have shown that the quantity used in framycetin-gramicidin nasal spray could hardly give rise to lung symptoms.

4 *Summary and conclusions.*

1 Thirty nurses and doctors and 20 patients who had been shown by repeated examinations (covering periods of 3 weeks to 6 months) to be nasal carriers of the same staphylococcal strain were treated with framycetin-gramicidin nasal spray 4 times daily for 7 and 3 days respectively.

Table 37 *Reappearance of staphylococci after treatment with framycetin-gramicidin nasal spray for three days*
(3 nasal carriers)

Pat. *no*	Day after *treatment*	No of bacteria (in thousands)					
		Nose	Throat	Upper lip	*Fingers*	*Hands*	Air contam
1	1	<0.04	<0.04	<0.02	<0.02	<0.5	<0.025
	2	1.60	<0.04	0.02	0.02	<0.5	0.025
	3	14.00	<0.04	0.02	0.02	<0.5	0.025
	6	84.00	0.04	0.44	0.08	4.0	<0.025
	7	184.00	<0.04	0.44	0.72	11.0	0.300
2	4	17.00	<0.04	<0.02	<0.02	<0.5	0.025
	5	36.00	<0.04	0.02	<0.02	<0.5	<0.025
	6	57.00	<0.04	<0.02	0.18	<0.5	0.100
	7	252.00	<0.04	<0.02	0.12	<0.5	0.500
	11	2 400.00	<0.04	5.60	3.60	12.0	4.900
3	1	<0.04	<0.04	<0.02	<0.02	<0.5	<0.025
	3	0.16	<0.04	<0.02	<0.02	<0.5	<0.025
	29	1,600.00	0.84	0.64	3.92	20.0	1.200
	30	800.00	0.36	0.40	1.78	1.5	0.300

bers of these organisms in the nose, axillae, vagina and faeces were also estimated.

Patients yielding perineal staphylococci on all 4 examinations were regarded as predominantly perineal carriers provided that they did not have staphylococcal lesions, more than 1 000 Staph. aureus in the samples from axillae, vagina and faeces, and more than 1 million Staph aureus in nasal samples. These criteria will be evident from the investigations referred to in chapters IV and VII. Fifteen patients fulfilled the conditions but 1 of them was too ill to be examined further.

The remaining 14 patients were examined 1—5 times in the course of 1—7 days. One patient (No. 13) was examined on 2 separate admissions 5 months apart.

Five of the 14 patients (Nos. 7, 11, 12, 13 and 14) were then treated morning and evening for 3 days by washing the perineum with 3 per cent hexachlorophane emulsion. Further samples were obtained the morning after completing treatment. One patient (No. 14) received treatment for further 3 days and was re-examined the next morning. Patient 13 was treated on both admissions to hospital.

The patients themselves undertook the treatment after preliminary instruction. The skin was first moistened with water. Hexachlorophane emulsion was then rubbed over the perineum and adjacent regions and washed off after 1 minute. The procedure was supervised to ensure correct technique. Other washing was performed with non-disinfectant soap.

In order to establish approximately equal experimental conditions before and after treatment, the patients were bathed and received clean clothes and bedclothes 2 days before the first pre-treatment examination and 2 days before the post treatment examination.

The methods of investigation are described in chapter II. All sampling fluids and media after treatment contained 1 per cent "Tween 80" to neutralize any residues of hexachlorophane emulsion that might be present.

2 *Results*

Quantitative estimations

The results of the examinations before treatment are given in table 38. The patients were divided into 3 groups according to the number of staphylococci on the perineum. Group 1 yielded less than 10^4 Staph. aureus in these samples, group 2 between 10^4 and 10^6 and group 3 more than 10^6 Staph. aureus.

Tables 39—42 give the correlation between perineal counts and counts from upper lip, fingers, hands and the skin just outside the perineum, respectively. Within wide limits the numbers of staphylococci in these samples increased with rising perineal counts.

Table 43 gives the correlation between the perineal counts and the staphylococcal aerial dissemination on bed making. It is evident that the ability to disperse the organisms into the air also increased with rising numbers on the perineum.

Five heavy dispersers were treated with hexachlorophane skin disinfection. Four of them showed a marked reduction of skin counts and aerial dissemination of staphylococci, from high pre-treatment values to negligible values the day after completion of treatment. The results of the examinations of one of them (patient 13 second hospital admission) given in fig. 16, provide a further illustration of this.

The fifth patient (No. 14) also showed a marked reduction in the number of staphylococci during treatment although the samples still yielded a few organisms

VI Perineal carriers of Staph. aureus examined before and after treatment with hexachlorophane

A. Previous investigations

The perineum was first suggested as a potential source and breeding place of Staph. aureus by Hare and Ridley (55). Gillespie et al. (41) isolated Staph. aureus from the perineum of 30 to 50 per cent of 2 to 10 day-old neonates. Ridley (107) obtained sufficient numbers of pathogenic staphylococci from the perineal area of 11 of 50 male students to class them as perineal carriers, 10 per cent having large numbers of these organisms on the perineum and very few of no nasal staphylococci. Tulloch et al. (127) examined the perineum of patients suffering from chronic furunculosis. 15 of 24 patients were perineal carriers of staphylococci. They suggested that this figure was probably higher than in the general population because perineal swabs were initially obtained from patients with boils on the lower part of the body. Boe et al. (19) examined 3,508 patients admitted to a medical ward. Thirteen per cent had Staph. aureus on the perineum, either alone (3 per cent) or combined with nasal and throat carriage.

The dispersal of staphylococci from perineal carriers has seldom been investigated (56, 57, 107). Perineal carriers, like nasal carriers, differ remarkably in their ability to disperse these organisms (57, 107) but the reason for this is unknown. On limited data it has been suggested that combined nasal and perineal carriers are liable to disperse larger numbers of staphylococci than pure nasal carriers (57, 107). Attempts to reduce the dissemination of Staph. aureus by perineal carriers have not been made.

In the present study the difference in ability of perineal carriers to disperse staphylococci was investigated. The possibility of reducing the number of staphylococci on the skin and their dispersal into the air by hexachlorophane (pHisoHex*) skin disinfection was also examined.

B. Personal investigations.

1. *Material and methods*

The material comprising 5 women and 9 men between 9 and 64 years of age was obtained in the following way. All patients (2,014 in all) admitted to The Medical Department B from August 1962 to October 1963 were examined for Staph. aureus in the nose, throat and on the perineum. About 13 per cent of the patients were perineal carriers on admission and these were examined for perineal staphylococci 4 times at intervals of 2—3 days. At the final examination the num-

*) Manufactured by The Winthrop Products Company, Surbiton, England.

Table 38 cont. *Skin contamination and aerial dissemination of staphylococci 14 perineal carriers.*
No. of bacteria (no. of colonies × dil. in thousands)

Pat. no., sex, age (yrs.)	Day of examin.	Perineum	Nose	Throat	Upper lip	Fingers	Hands	Extra-perineal area	Air contamination (bed making)
11 M 63	1	640	0.04	<0.01	<0.02	7.82	26.5	16.00	24 700
12 M 23	1	1,000	12.00	0.76	<0.02	0.96	84.0	7.00	1,300
	3	2,560	16.00	0.88	<0.02	26.80	50.0	3.00	16,700
	4	1,840	16.00	0.60	<0.02	9.68	16.5	16.00	14 100
13 M 14	1	2,240	20.00	<0.01	1.48	53.00	210.0	36.00	51,900
	2	8,160	149.00	0.04	5.28	33.00	66.0	68.00	56,900
	3	4,320	*61.00*	*<0.04*	*0.40*	*11.00*	*120.5*	*104.00*	52,300
	1	18,200	12.00	0.04	1.24	287.00	1 710.0	64.00	111,200
	2	15,720	4 00	0.04	8.48	126.00	360.0	4 4.00	114,800
	3	26,520	8.00	<0.04	1.56	670.00	1,320.0	920.00	210 400
	4	12,400	1._00	<0.04	0.36	128.00	420.0	528.00	144 900
	5	11,280	16.00	<0.04	6.48	51.00	840.0	160.00	131,800
14 M 60	1	10,720	64.00	<0.04	0.08	64.00	180.0	36.00	200,400
	2	24 400	160.00	<0.04	1.08	61.00	730.0	21.00	206,700

Table 39 *Correlation between staphylococcal counts from perineum and upper lip 14 perineal carriers.*

Perineal count	No. of examinations	Upper lip			
		< 20 bact./sample		> 1,000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$	10	8	80.0	0	0.0
10^4-10^6	14	10	71 4	1	7 1
$>10^6$	13	3	23.1	7	53.8
Total	37	21	56.8	8	21.6

Table 38 *Skin contamination and aerial dissemination of staphylococci 14 perineal carriers. No of bacteria (no. of colonies × dil. in thousands)*

Pat. no. sex, age (yrs.)	Day of examin.	Perineum	Nose	Throat	Upper lip	Fingers	Hands	Extra perineal area	Air contamination (bed making)
1 F 26	1 2	4 1	80.00 92.00	0.04 <0.04	<0.02 <0.02	<0.02 <0.02	<0.5 <0.5	<0.08 <0.08	<0.025 <0.025
2 M 23	1 2	2 9	0.04 0.08	<0.04 <0.04	<0.02 <0.02	0.04 <0.02	<0.5 <0.5	<0.08 <0.08	<0.025 <0.025
3 M 21	1 2	2 6	48.00 28.00	<0.04 <0.04	0.04 0.02	<0.02 0.02	<0.5 0.5	<0.08 <0.08	0.100 0.100
4 M 16	1 2	2 5	4.00 20.00	0.08 <0.04	<0.02 <0.02	0.04 0.04	<0.5 <0.5	0.08 0.40	0.100 0.100
5 F 46	1 2 5	80 280 44	<0.04 <0.04 <0.04	0.04 <0.04 <0.04	<0.02 0.02 <0.02	<0.02 0.02 0.02	<0.5 1.0 <0.5	0.16 0.08 <0.08	3.300 2.500 0.900
6 F 53	1 4 5	444 960 400	120.00 148.00 16.00	<0.04 <0.04 <0.04	<0.02 <0.02 <0.02	0.02 0.14 <0.02	<0.5 <0.5 <0.5	1.68 <0.08 4.00	1.800 1.500 0.200
7 F 49	1 6 7	464 920 160	1.04 0.16 <0.04	<0.04 0.04 <0.04	<0.02 0.08 <0.02	0.18 4.56 0.18	2.5 40.0 6.0	44.00 4.00 6.00	3.000 2.400 1.700
8 F 10	1 3	351 891	16.00 4.00	1.52 3.42	<0.02 1.54	4.18 10.56	18.0 105.0	1.04 0.88	3.700 35.400
9 M 33	1 4	8 440	<0.04 20.00	<0.04 <0.04	<0.02 0.02	<0.02 4.12	<0.5 12.0	<0.08 0.32	0.025 13.900
10 M 27	1 5	3 252	<0.04 0.04	<0.04 <0.04	<0.02 <0.02	<0.02 2.82	<0.5 11.5	<0.08 2.40	<0.025 7.900

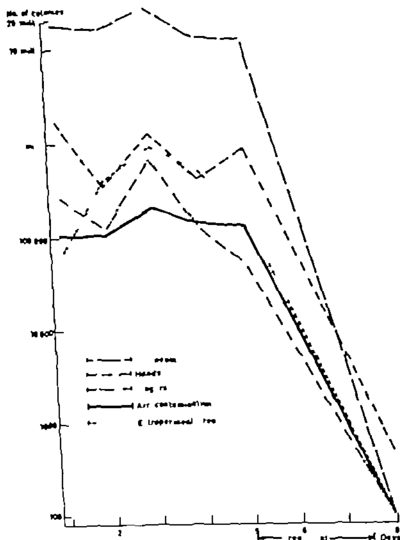


Fig. 16. Staphylococcal counts from patient 13 before and after treatment with hexachlorophane emulsion.

(fig. 17) The patient was treated for further 3 days after which, samples from the perineum and adjacent area and from the fingers and hands, no longer yielded Staph. aureus and only 20 bacteria were isolated from the upper lip. The aerial dissemination was also minimal (100 bacteria)

Patient 13 yielded no staphylococci in perineal cultures on discharge after the first stay in hospital but, as seen in table 38 larger quantities were demonstrated 5

Table 40 *Correlation between staphylococcal counts from perineum and fingers. 14 perineal carriers*

Perineal count	No. of examinations	Fingers			
		<20 bact./sample		>1 000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$	10	6	60 0	0	0 0
10^4-10^6	14	3	21 4	6	42.9
$>10^6$	13	0	0 0	12	92.3
Total	37	9	24.3	18	48.6

Table 41 *Correlation between staphylococcal counts from perineum and hands 14 perineal carriers*

Perineal count	No. of examinations	Hands			
		<500 bact./sample		>10 000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$	10	9	90.0	0	0.0
10^4-10^6	14	5	35 7	3	21 4
$>10^6$	13	0	0.0	13	100 0
Total	37	14	37.8	16	43.2

Table 42 *Correlation between staphylococcal counts from perineum and extra-perineal area. 14 perineal carriers.*

Perineal count	No. of examinations	Extra perineal area			
		<80 bact./sample		>10 000 bact./sample	
		No	Per cent	No	Per cent
$<10^4$	10	8	80.0	0	0.0
10^4-10^6	14	2	14.3	2	14.3
$>10^6$	13	0	0.0	10	76.9
Total	37	10	27 0	12	32 4

Table 43 *Correlation between staphylococcal perineal counts and degree of aerial dissemination on bed making 14 perineal carriers*

Perineal count	No. of examinations	Degree of air contamination			
		<100 bact. /sample		>10,000 bact. /sample	
		No.	Per cent	No.	Per cent
$<10^4$	10	6	60.0	0	0.0
10^4-10^6	14	0	0.0	3	21 4
$>10^6$	13	0	0 0	12	92.3
Total	37	6	16.2	15	40.5

Staph. aureus particles.

strains demonstrated 3 patients had 2 strains. *Ten strains were resistant to 1 or* more antimicrobial agents. All 10 were resistant to penicillin 6 to sulphathiazole, 5 to streptomycin, 5 to tetracycline 3 to erythromycin and 2 to chloramphenicol. Table 44 gives the results of antibiogram determinations and phage typing in relation to perineal counts. The frequency of resistant strains was greatest for patients with th highest counts. The results of the examinations of patients 9 and 10 ar included in group 2.

On phage typing, all trains proved typable, the majority being demonstrated in phage group III There were no strains in phage groups IV and V

Thirteen patients yielded positive nasal cultures nd 7 positive throat cultures. Ten patients had identical strains in the nose and on the perineum, nd 4 n the throat and perineum.

Despite the exclusion, at preliminary examinations of perineal carriers with more tha 1,000 staphylococci per gram of faeces, 2 patients (Nos 3 and 11) yielded 1,000 and 12,000 bacteria respectively i these samples. The perineal and faecal strains were identical The remaining patients had less than 1 000 staphylococci per gram of faeces. Samples from the axillae and vagina were negative (less than 1 000 staphylococci)

Antibiogram determinations and phage typing were performed on 21 colonies from the upper lip 72 from the fingers, 49 from the hands and 30 from the region adjacent to the perineum — 175 colonies altogether The reactions of 172 colonies to the antimicrobial agents and phages were identical with those of the perineal strains, or varied so slightly that they were assumed not to be different Phage typing did not determine whether the remaining 3 colonies were different from the respective perineal strains but serological typing revealed that these strains were most probably identical

Antibiogram determinations and phage typing were performed on 173 colonies from the air samples. On comparison with the espective skin strains, 170 colonies gave identical reactions, or reactions that varied so slightly that the trains could not be classified as different The remaining 3 colonies were demonstrated in samples from patients 3 5 d 7 and were different from the other strains harboured by these patients.

3. *Discussion*

An important problem the present investigation was whether the staphylococci on the upper lip fingers, hands, area round the perineum and in the air samples were really due to dispersal from the perineum and not to contamination from the en-

T ble 44 *Drug sensitivity and phage grouping of perineal strains in elation to perineal count.*

Perineal count	No. of strains					
	Total	Resistant	Phage groups			
			I	II	III	Miscellaneous
<10⁴	5	2	2	1	1	1
10⁴–10⁶	8	5	1	0	6	1
>10⁶	4	3	1	0	1	2
Total	17	10	4	1	8	4

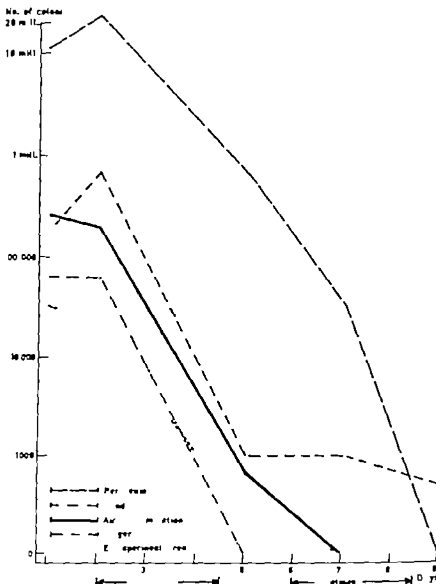


Fig 17 Staphylococcal counts from patient 14 before during and after treatment with hexachlorophane emulsion

months later than at the first admission The strains were identical on both occasions. The perineal count from patient 14 was small the day after completion of treatment but increased during the following 7 days the strain being identical with that previously demonstrated

The number of staphylococci in the vestibule of the nose was unchanged by the treatment.

No side-effects were observed On the other hand, 2 patients (Nos 13 and 14) suffered from perineal pruritus which disappeared during treatment

Antibiogram and phage patterns

On the average, antibiogram determinations and phage typing were performed on 5 perineal colonies from each patient. Sixty nine colonies were examined and 17

2. The numbers of staphylococci on the upper lip fingers, hands and the region around the perineum increased with rising perineal counts. This also applied to aerial dissemination of staphylococci on bed making

3 The heaviest dispersers among perineal carriers dispersed much larger numbers of staphylococci into the air than nasal carriers (chapter IV) Perineal carriers may therefore be a greater problem in the control of hospital infection than their frequency would suggest.

4 Five perineal carriers were treated by washing the perineum and adjacent areas with hexachlorophane emulsion. The number of staphylococci on the skin and the aerial dissemination fell from high pretreatment values to practically nil after completing treatment.

Side-effects of treatment were not observed.

Washing with hexachlorophane emulsion is therefore regarded as a valuable measure for controlling the dispersal of staphylococci from perineal carriers.

vironment or dissemination from nose and throat. As the strains in air and skin samples were identical with the respective perineal strains contamination from the surroundings must have been insignificant. Apparently dispersal from the nose and throat were also of minor importance. The results reported in chapter IV showed that individuals whose nasal counts did not exceed those of the 14 perineal carriers dispersed far fewer staphylococci and that throat carriers dispersed even less In addition 5 patients had different strains in nose and throat from those on the perineum, the former not being demonstrated in skin and air samples. The 14 perineal carriers did not have other staphylococcal multiplication sites of any significance (lesions axillae vagina faeces) Apparently most of the organisms on the upper lip fingers hands, area round the perineum and in the air samples were due to dispersal from the perineum.

The fact that some perineal carriers are heavy dispersers and others light, must, according to the present results mainly be due to the number of staphylococci on the perineum even though individual variations are quite large. The results of the examinations of patients 9 and 10 support this view These patients had few perineal staphylococci on the first examination but on the second examination some days later the numbers had risen considerably and dispersal to other skin areas and into the air was also markedly greater than before.

In contrast Ridley (107) found little correlation between the numbers of staphylococci on the perineum and in samples of skin and air contamination. However he did not examine his patients under standardized conditions and his technique allowed only a crude classification of the bacterial counts. The large individual variations may therefore have masked a possible correlation. On the other hand, Ridley stated that there was usually good correlation between contamination of the fingers and clothes and the degree of dispersal into the air

The perineal carriers had fewer staphylococci on the upper lip fingers and hands than the nasal carriers (chapter IV) but they were capable of dispersing much larger numbers into the air Patients 13 and 14 for example, dispersed as many staphylococci into the air as all the 100 nasal carriers in chapter IV together Apart from contamination via the hands, direct transfer of staphylococci probably takes place from the perineum and surrounding skin to the bedclothes This pronounced capacity of perineal carriers to disperse the organisms into the air shows that the crux of the staphylococcal problem may not lie solely in nasal carriage. This is probably one of the reasons why treatment of nasal carriers with antibiotic nasal spray has failed to reduce the post-operative infection rate.

Treatment of perineal carriers with hexachlorophane skin disinfection very effectively reduced the number of staphylococci on the skin and dispersal into the air This is in accordance with the considerable reduction of the skin flora following the use of hexachlorophane liquid cream (9 49 83 85 118) In maternity departments, washing infants with hexachlorophane emulsion has also considerably reduced the frequency of nasal carriers and staphylococcal lesions (64 101) Hexachlorophane emulsion is therefore a valuable measure for reducing the dispersal of staphylococci from perineal carriers.

4 Summary and conclusions

1 Staphylococcal skin counts and aerial dispersal on bed making were investigated in 14 patients who were mainly perineal carriers.

for 2—3 consecutive days. In 14 of the 15 patients, the lesions were washed morning and evening with 3 per cent hexachlorophane emulsion (pHisoHex) and the patients were re-examined during and after treatment

After preliminary instruction the patients carried out the treatment themselves. A procedure similar to that described in chapter VI was employed. Those with perineal staphylococci treated the perineum as well, the rest of the body being washed with ordinary non-disinfectant soap Three patients (Nos. 1 2 and 12) were unable to manage the treatment themselves and were therefore washed by ward nurses. The patient with auditory meatal eczemas was treated with tampons moistened in a solution of 10 ml hexachlorophane emulsion in 90 ml water Four to 30 ml hexachlorophane emulsion were used at each treatment, depending on the size of the skin lesion

Two patients (Nos. 4 and 11) were also treated daily with framycetin-gramicidin nasal spray and one patient (No. 2) with fucidic acid 0.5 g × 3 Apart from these instances, chemotherapeutic or skin disinfecting agents other than hexachlorophane emulsion were not used

To achieve approximately equal experimental conditions before, during and after treatment, the patients were bathed or washed on a stretcher and received clean clothes and bedclothes 2 days before the first pre-treatment examination and again 2 days before each examination during and after treatment.

The investigation methods are described in chapter II Three patients (Nos. 1 2 and 12) had such extensive skin lesions that only a 25 sq cm area was examined From the remaining 12 patients, the samples were obtained from the whole affected skin area. The sampling fluids and media used during and after treatment contained 1 per cent 'Tween 80' in order to neutralize possible residues of hexachlorophane emulsion.

2 *Results*

Quantitative estimations

Table 45 gives the results of the examinations before, during and after treatment. Before treatment the dispersal of staphylococci into the air from the 2 patients with extensive pyodermias was about 3 times as great as for all the other patients together Calculated on the basis of the mean counts, patient 1 had more staphylococci on the fingers and hands than all the other patients together The 4 patients with infected hand sores yielded large counts from the hands, but air contamination was somewhat less than for the patients with infected eczemas in the pubic, perineal and inguinal regions. Considerable numbers of staphylococci were isolated from the lesions in patients 13 14 and 15 but air contamination was moderate.

Fourteen patients were treated by washing with hexachlorophane. In all these, there was a marked reduction in staphylococcal counts, from high pretreatment values to very modest numbers during the treatment and after its completion. To illustrate this relationship three patients will be discussed in detail

Fig 18 gives the results of treatment of patient 1 Before therapy the patient constantly developed yellow-green bullae which burst and dried into crusted sores up to 5 × 5 cm. After treatment was instituted this process ceased and 4 days later the skin and air contamination were considerably reduced However the number of staphylococci in these samples did not fall to modest levels until 11 days after the commencement of treatment. Therapy was

VII The effect of hexachlorophane treatment on self-contamination and dispersal of Staph aureus by patients with staphylococcal dermal lesions

A Previous investigations

Patients with staphylococcal lesions are regarded as dangerous carriers The lesions are often caused by strains resistant *to several antibiotics and presumably* more virulent than sensitive organisms (7) Further it has been shown that indi*viduals with staphylococcal lesions have* probably been the source of infections in maternity units (5 34) and in medical and surgical wards (6 94 124)

Patients with widespread staphylococcal infected skin lesions are among the heaviest dispersers (26 56) Thomas and Griffiths (125) found that the air of wards housing patients with skin diseases contained considerably greater numbers of staphylococci than the air of other wards

We know that patients with staphylococcal infected skin lesions can disperse large numbers of Staph. aureus, but little is known as to how effectively this dispersal can be reduced In the present investigation, the skin contamination and aerial dispersal rate of patients with staphylococcal dermal lesions were determined before and after hexachlorophane treatment

B Personal investigations

1 *Material and methods*

The material consists of 9 women and 6 men between the ages of 17 and 83 years *Two patients (Nos. 1 and 2) had pyoder*mias involving both thighs buttocks and the lower part of the back and abdomen *The lesions covered about 30—40 per cent* of the total skin surface. Five patients (Nos. 3 4 5 6 and 7) had small infected *eczemas in the perineal* inguinal and pubic regions, four (Nos 8, 9 10 and 11) had small sores on the hands and one (No 10) also on the right leg One patient (No 12) was examined on two separate admissions to hospital. On the first occasion she had furuncular residua on the back, and on the second considerable numbers of staphylococci were demonstrated in the left axilla although there was no visible lesion. The remaining three patients had various minor skin lesions, such as infected eczemas in both auditory meatus (No 13) hidrosadenitis in both axillae (No 14) and infected atheroma on the left leg (No 15)

All patients were examined once daily

ble 43 cont. *Skin contamination and aerial dissemination of staphylococci in relation to treatment (hexachlorophane emulsion) 15 patients with staphylococcal dermal lesions. No. of bacteria (no. of colonies × dil. in thousands)*

t. no., sex, age (yrs.)	No. of day treated	Day of treat ment	Dermal lesion	Nose	Throat	Upper lip	Fingers	Hands	Peri-neum	Air contamination (bed making)
		+ 3	320.00	120.00	0.32	0.16	7.80	1 470.0	<1.00	8.900
8		− 2		668.00	0.64	0.92	0.24	1,020.0	<1.00	3.500
F	5	− 1		40.00	8.00	0.44	11.84	1,040.0	<1.00	4.900
18		3	<0.04	112.00	208.00	<0.02	<0.02	<0.5	<1.00	0.200
		+ 1		332.00	4.80	1.52	0.02	0.5	<1.00	0.0.0
9		− 3	840.00	324.00	<0.04	0.04	1 41	1,200.0	<1.00	13.200
M	5	− 2		228.00	<0 04	1.96	2.48	2,080.0	<1.00	17.000
42		+ 1	320.00	344.00	<0.04	0.08	2.64	880.0	<1.00	10.900
		4	<0.04	800.00	<0.04	<0.02	0.02	<0.5	<1.00	0.100
10		+ 2	2.00	3.00	<0.01	0.02	93.00	320.0	<1.00	2.600
F	5	− 1	1.64	4.00	<0.04	0.02	41.00	160.0	<1.00	3.500
22		3	<0.02	8.00	<0.04	0.04	<0.02	0.5	<1.00	0.025
11		+ 6		2,920.00	0.08	0 72	0.36	172.0	<1.00	3.500
F	4	+ 1	102.00	<0.04	0.04	<0.02	0.80	236.0	<1.00	2.500
55		2	<0.04	<0.04	0.04	<0.02	<0.02	<0.5	<1.00	<0.025
		+ 2	<0.04	<0.04	0.04	<0.02	<0.02	<0.5	<1.00	<0.025
		− 4	0.84	152.00	0.08	0.06	0.30	<0.5	<1.00	13.300
	5	− 3	0.30	168.00	<0.04	0.46	0.22	7.0	<1.00	10.200
12		− 1	0.36	64.00	0.04	0.02	0 40	<0.5	<1.00	10.000
F		3	0.04	60.00	0.08	<0.02	0 12	<0.5	<1.00	<0.025
24		− 3	8.40	200.00	0.04	1 12	0.20	5.0	<1.00	0.700
	—	− 2	16.80	280.00	0.04	<0.02	<0.02	1.0	<1.00	1.300
		1	52.80	80.00	0.04	0.12	0.36	4.0	<1.00	0.700
13		2	10 400.00	20.00	<0.04	0.04	1.92	140.0	<1.00	4.800
F	3	− 1	20,400.00	20.00	0.08	<0.02	3 46	100.0	<1.00	3.900
72		3	<0.04	188.00	0.04	<0.02	0.08	<0.5	<1.00	0.100
14		+ 2	46,400.00	8.00	0 12	<0.02	0.32	6.5	<1.00	2.300
F	—	− 1	120800.00	8.00	<0.04	<0.02	0.12	12.5	<1.00	2.000
63										
15		− 3	11,200.00	1.00	<0.04	2.24	1.60	340.0	<1.00	3.300
M	6	− 1	4 760.00	0.40	<0.04	2.80	0.24	80.0	<1.00	7.200
56		5	<0.04	0.08	0.44	<0.02	<0.02	<0.5	<1.00	<0.025

From lesion on ight leg

e 45. *Skin contamination and aerial dissemination of staphylococci in relation to treatment (hexachlorophane emulsion) 15 patients with staphylococcal dermal lesions. No of bacteria (no of colonies × dil. in thousands)*

no., x, ge s.)	No. of days treated	Day of treat ment	Dermal lesion	Nose	Throat	Upper lip	Fingers	Hands	Peri- neum	Air contamination (bed making)
1 F 4	25	− 3	2 080 00	408.00	2 40	2 60	1 610 00	8,056 0	2,800 00	1 049.960
		− 2	6,480.00	488 00	2.80	0 76	1 140 00	6,240.0	4 440 00	1,353.240
		− 1	2 960 00	1,240.00	2.00	0.28	710 00	1 080.0	1 880 00	997.500
		4	320 00	1 872.00	3.20	3.24	16.00	120 0	240.00	99.120
		8	8.00	618.00	7.20	0.34	2 42	24.0	<0 08	15.120
		11	<0 08	602 00	8.80	0 12	0 48	1.5	<0.08	0.840
2 F 0	9	− 2	280 00	236.00	1.56	2 80	100 00	842.0	2,840 00	1 113.420
		− 1	810.00	288 00	1.32	3 64	92 00	680 0	9 680 00	1 170.120
		3	<0.08	256 00	0 08	0.08	5 00	28.0	80.00	15.540
		5	<0.08	320 00	0.96	0 04	0 08	<0.5	16 00	0.840
		7	<0 08	<0 04	<0 04	<0.02	0 04	<0.5	0 40	0.420
		9	<0 08	<0 04	<0.04	<0 02	<0 02	<0.5	<0.08	<0.420
		+ 8	<0 08	0.24	<0 04	<0 02	<0 02	<0.5	<0 08	<0.420
		+17	<0.08	316 00	0.92	0 44	0.30	21 0		3 700
3 '	4	− 2	1 840 00	183.00	0 12	0 02	3 48	80.0	480.00	72.800
		− 1	840 00	641.00	0.52	0 66	8.44	112 0	368 00	70 400
		2	5.52	1,368.00	0.24	0 08	0.20	<0.5	<0 08	0 700
		4	<0 08	248.00	2 16	<0 02	<0 02	<0.5	<0.08	0.100
	5	− 3	5 240.00	368 00	<0 04	0 06	4 00	36 0	304 00	145.400
		− 2	964 00	524 00	<0.04	0.42	33.20	130.0	355 00	132 400
		− 1	3,120 00	0 60	<0 04	0.24	60 00	138 0	836 00	200.900
		2	4 00	0 08	0 12	<0 02	0.12	1 0	<0 08	3.500
		4	0.80	<0 04	0.34	<0.02	0 12	<0.5	<0.08	0.500
		6	0 08	<0 04	0 18	<0.02	<0.02	<0.5	<0.08	<0.025
	9	− 3	680 00	224 00	0.24	3 40	63 00	40 0	96.00	17.300
		− 1	320.00	400.00	7.28	0 44	25 00	26 0	72.00	21 400
		4	<0 08	92.00	8.00	<0 02	<0 02	<0.5	<0 08	<0.025
	2	− 2	4 120 00	920 00	0 12	0.08	4 00	20.0	360.00	31.000
		− 1	1 040 00	280 00	0.24	<0 02	4.08	210 0	92.00	32.000
		2	5 60	1 400.00	<0 04	<0 02	0 16	1.5	4 00	2 000
	18	− 3	8,240.00	60 00	0 04	9.00	5.32	60 0		29.900
		− 2	4 840 00	160.00	0 16	1 00	10.56	140 0		37 400
		− 1	2 440 00	40.00	<0 04	0 16	2 18	20 0		38.600
		4	16.00	160 00	<0.04	0.0[illegible]	0.08	<0.5		0.500

before treatment. + = after treatment.

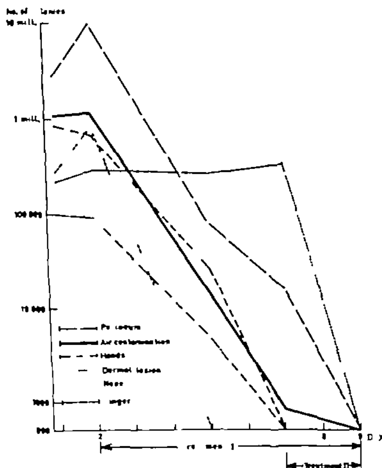


Fig. 19 Staphylococcal counts from patient 2 before, during and after treatment with hexachlorophane emulsion (I) and fucidic acid (II)

2 days treatment with hexachlorophane no staphylococci were demonstrated in skin and air samples.

For the remaining patients, reduction in the staphylococcal counts to minimal values was bserved after only 2—5 days treatment. The skin lesions had begun to heal. Patients with pruritus of the affected skin areas noticed considerable improvement after only 1—2 days treatment. Therapy was continued until the lesions were healed this took 6 to 25 days.

Patients 4 and 11 were treated with framycetin-gramicidin nasal spray 4 times daily for 1 and 4 days respectively before they received hexachlorophane treatment. After spray therapy the nasal counts fell practically to nil, while the skin and air samples yielded bout the same number as before treatment.

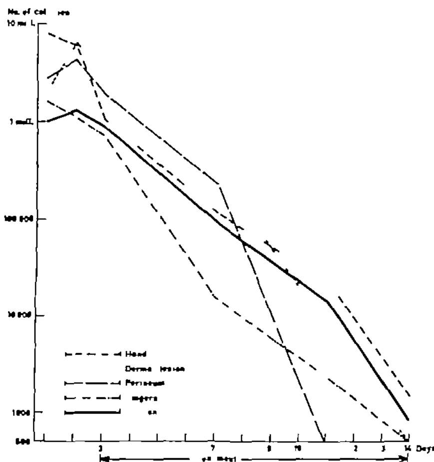


Fig 18 Staphylococcal counts from patient 1 before, during and after treatment with hexachlorophane emulsion.

continued and the patient was discharged 14 days later The affected skin area was healed The nasal and throat counts were *about the same as before treatment.*

Fig 19 gives the results of treatment of *patient 2. After 3 days therapy skin and* air contamination were considerably reduced and 2 days later only small numbers of staphylococci were demonstrated in these samples From then on the patient also received fucidic acid therapy for 4 days. During this treatment the staphylococci in nasal and throat cultures also disappeared and on the 9th day of treatment all samples were negative and the skin lesion was almost healed Therapy was then stopped After 8 and 17 days rising counts were demonstrated in nasal cultures, and skin and air samples yielded *a few organisms. The skin lesion had* healed

Fig 20 gives the results of treatment of patient 5 who had acute hepatitis. He sweated profusely and suffered intense pruritus. Seven days after admission he developed a staphylococcal-infected eczema in the pubic region Identical strains were isolated from the eczema as had been isolated from the nose and perineum at the time of admission The patient had a different strain in his throat. He was a marked staphylococcal disperser but after

nasal samples and only 1 differed from the skin lesion strains. Of the 14 strains isolated from the throat, 3 differed from the skin lesion strains.

Six patients had positive perineal samples, identical strains being isolated from perineum and lesion. Two patients (Nos. 1 and 4) had positive faecal samples. These yielded 5 000 and 24 000 staphylococci per gram of faeces respectively the strains being identical with those from the skin lesions.

Antibiogram determinations and phage typing were performed on 46 colonies from the upper lip 108 from the fingers, 74 from the hands and 283 from air samples. Altogether 511 colonies were examined. When compared with the strains from the skin lesions, the reactions of 504 colonies differed so slightly that they were assumed to be identical. Serological typing showed that 4 of the remaining 7 colonies were most probably identical with the skin lesion strains 3 colonies being different. These 3 colonies were isolated from air samples from patients 12 and 13 and were not included in the calculation of all contamination.

Eight of the 15 patients already had their lesions at the time of admission to the department but the other 7 acquired their lesions after admission. In these 7 patients, the skin lesion strains were identical with those isolated from nose, throat or perineum before the lesions became manifest.

3 *Discussion.*

An essential problem in this study was whether the staphylococci on the upper lip, fingers and hands and in the air samples really were due to dispersal from the skin lesions and not to contamination from the environment or dispersal from nose throat or perineum. As the strains from the lesions and those from the other skin areas and in air samples were identical, contamination from the environment must have been insignificant.

The results presented in chapter IV showed that nasal carriers who did not yield more staphylococci from the nasal vestibule than the 15 patients with skin lesions dispersed far fewer organisms, the throat carriers dispersing even less. In addition 4 of the 15 patients had strains in the nose or throat different from those in the lesions and the former strains were not demonstrated in skin and air samples. On the other hand a somewhat larger proportion of the bacteria from fingers, hands and in air samples from the 6 patients who were also perineal carriers, was probably due to perineal dispersal. However on the basis of the results presented in chapter VI it is reasonable to assume that the majority of the staphylococci in the samples from these patients were due to dispersal from the lesions.

Patients with widespread pyodermias can disperse much greater numbers of staphylococci than patients with small skin lesions, or nasal and perineal carriers. Patient 1 for example, had larger staphylococcal counts from fingers and hands than all the other patients together and also dispersed greater numbers into the air The 100 nasal carriers previously mentioned (chapter IV) taken together had about the same number of staphylococci on the fingers and hands as patient 1 The aggregate count from these skin areas of the 14 perineal carriers mentioned earlier (chapter VI) was lower than that of patient 1 Patients 1 and 2 dispersed more staphylococci into the air than the other patients with lesions, the 14 perineal carriers and the 100 nasal carriers altogether Air samples from patient 1 yielded about 500 times as many colonies as the mean value for air samples from the 100 nasal

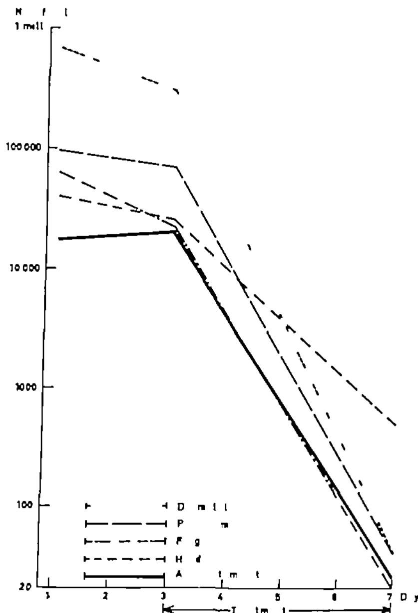


Fig 20 Staphylococcal counts from patient 5 before and after treatment with hexachlorophane emulsion.

Antibiogram and phage patterns

Antibiogram determinations and phage typing were performed on 71 staphylococcal colonies from the lesions. Altogether 18 strains were demonstrated. Two patients had 2 and 3 strains respectively. Ten strains (56 per cent) were resistant to penicillin and tetracycline.

Sixteen strains were lysed by the phages used. Five strains belonged to phage group III, 4 strains to phage group I and 4 strains to the miscellaneous group. There were 1 and 2 strains respectively in phage groups II and V.

All patients had positive nasal cultures and 13 had positive throat cultures. Eighteen strains were demonstrated in the

nasal samples and only 1 differed from the skin lesion strains. Of the 14 strains isolated from the throat, 3 differed from the skin lesion strains.

Six patients had positive perineal samples, identical strains being isolated from perineum and lesion. Two patients (Nos. 1 and 4) had positive faecal samples. These yielded 5 000 and 24 000 staphylococci per gram of faeces respectively, the strains being identical with those from the skin lesions.

Antibiogram determinations and phage typing were performed on 46 colonies from the upper lip, 108 from the fingers, 74 from the hands and 283 from air samples. Altogether 511 colonies were examined. When compared with the strains from the skin lesions, the reactions of 504 colonies differed so slightly that they were assumed to be identical. Serological typing showed that 4 of the remaining 7 colonies were most probably identical with the skin lesion strains, 3 colonies being different. These 3 colonies were isolated from air samples from patients 12 and 13 and were not included in the calculation of air contamination.

Eight of the 15 patients already had their lesions at the time of admission to the department but the other 7 acquired their lesions after admission. In these 7 patients, the skin lesion strains were identical with those isolated from nose, throat or perineum before the lesions became manifest.

3. *Discussion.*

An essential problem in this study was whether the staphylococci on the upper lip, fingers and hands and in the air samples really were due to dispersal from the skin lesions and not to contamination from the environment or dispersal from nose, throat or perineum. As the strains from the lesions and those from the other skin areas and in air samples were identical contamination from the environment must have been insignificant.

The results presented in chapter IV showed that nasal carriers who did not yield more staphylococci from the nasal vestibule than the 15 patients with skin lesions dispersed far fewer organisms, the throat carriers dispersing even less. In addition, 4 of the 15 patients had strains in the nose or throat different from those in the lesions and the former strains were not demonstrated in skin and air samples. On the other hand, a somewhat larger proportion of the bacteria from fingers, hands and in air samples from the 6 patients who were also perineal carriers, was probably due to perineal dispersal. However, on the basis of the results presented in chapter VI, it is reasonable to assume that the majority of the staphylococci in the samples from these patients were due to dispersal from the lesions.

Patients with widespread pyodermias can disperse much greater numbers of staphylococci than patients with small skin lesions, or nasal and perineal carriers. Patient 1, for example, had larger staphylococcal counts from fingers and hands than all the other patients together and also dispersed greater numbers into the air. The 100 nasal carriers previously mentioned (chapter IV) taken together had about the same number of staphylococci on the fingers and hands as patient 1. The aggregate count from these skin areas of the 14 perineal carriers mentioned earlier (chapter VI) was lower than that of patient 1. Patients 1 and 2 dispersed more staphylococci into the air than the other patients with lesions, the 14 perineal carriers and the 100 nasal carriers altogether. Air samples from patient 1 yielded about 500 times as many colonies as the mean value for air samples from the 100 nasal

carriers. Although individual nasal and perineal carriers may disperse considerable numbers of staphylococci, patients with widespread staphylococcal infected dermal lesions are far heavier dispersers. Further 10 (56 per cent) of 18 strains isolated from the lesions were resistant to penicillin and tetracycline in contrast to only 18 per cent of the strains from the nasal carriers (chapter IV)

Patients with lesions small enough to be covered by bandages or so located that they do not come into direct contact with the bedclothes disperse relatively small numbers of staphylococci. Patients 13 14 and 15 for example, dispersed few staphylococci into the air in relation to the large numbers demonstrated in samples from the lesions. The first patient had infected eczema of both auditory meatus. Secretion from the eczema was negligible. The sheltered site of the lesions probably prevented direct contact with the bedclothes and thus reduced the dispersal into the air The other two patients used bandages on their lesions.

Washing with hexachlorophane emulsion must be regarded as a valuable treatment for reducing or preventing the dispersal of staphylococci from patients with infected skin lesions. Patients who were heavy dispersers were almost converted to non-dispersers after a few days of this treatment alone, and their skin lesions began to heal Patients who were also nasal carriers had remarkably few staphylococci on fingers and hands during treatment though they still had large numbers in nasal cultures. Hexachlorophane therapy probably also reduces the contamination of the hands with nasal staphylococci

Autoinfection probably plays an important part in the epidemiology of staphylococcal infections. All the 7 patients who acquired their infections while in hospital were most probably infected from their own carrier sites and not from the environment. In all these cases the strain causing the infection was identical with that demonstrated in the nose, throat or on the perineum at the time of admission or in later samples obtained before the lesions developed It has been suggested in a series of earlier investigations that many staphylococcal infections are due to autoinfection with organisms from the nasal vestibule (23 27 28 65 130 140, 148)

Staphylococcal lesions on the lower half of the body are probably often due to infection with organisms from the perineum. Four of the 7 patients who developed their lesions in hospital had identical strains on the perineum on admission as were later demonstrated in their lesions. These patients developed lesions in the inguinal and pubic regions or on the buttocks and lower half of the abdomen and back. The other 3 patients had abundant nasal staphylococci but negative perineal samples, and only one of them developed infections below the waist. Tulloch et al (127) also found that perineal carriage was sometimes associated with boils on the trunk, buttocks and thighs The results of the present study indicate that the perineum may quite often be the source of infection for lesions on the lower part of the body as mentioned by Kay (74) and that the rest of the body is most frequently infected from the nose.

4 *Summary and conclusions*

1 The numbers of Staph aureus on various skin sites and the ability to disperse the organisms into the air on bed making were investigated in 15 patients with staphylococcal skin lesions. Two patients had extensive pyodermias. One patient had bilateral axillary hidrosadenitis and the other had small infected eczemas, sores, or furuncular residua

2. Greater numbers of staphylococci were dispersed into the air from each of the patients with pyodermias than from the other 13 patients together One of the pyodermia patients also yielded more staphylococci on the fi gers and hands than the other 14 patients together

Patients with widespread staphylococcal-infected skin lesions are "heavy dispersers" of staphylococci. As their strains are often resistant to several antibiotics they should also be regarded as "dangerous carriers"

3 Fourteen patients were treated by washing their skin lesions with hexachlorophane emulsion. In the course of 2—11 days, all were converted from heavy dispersers lmost to "non-dispersers and at the same time the lesions began to heal

No side-effects of treatment were observed.

Washing with hexachlorophane emulsion is regarded as a valuable measure for preventing staphylococcal dispersal from patients with staphylococcal skin lesions.

4 Seven of the 15 patients developed their lesions while in hospital. In all these patients, the strains causing the lesions were identical with those demonstrated in the nose, throat or on the perineum before the lesions developed. It is assumed that the patients were infected with organisms from their own carrier sites and that the perineum may quite often be the source of infections on the lower part of the body

VIII General summary and conclusions

I The purpose of the present investigation *was primarily to study why staphylococcal* carriers differ in their ability to disperse their organisms into the air and secondly to investigate the effect of antibiotic nasal spray and hexachlorophane skin disinfection in reducing the staphylococcal dispersal from individual carriers

II To study these problems suitable methods of quantitative estimations of staphylococci on different sites of the body were evaluated To assess the ability of individuals to disperse staphylococci into the air a standardized form of bed making in a test room was developed

The capacity to coagulate rabbit plasma was used as the only criterion for the selection of pathogenic staphylococci. Antibiogram determinations phage typing and serological typing were used to determine *whether staphylococcal strains isolated* from different sites of an individual were identical

III The design of experiments is described

Staphylococcal carriers among the patients admitted to Medical Department B Haukeland Hospital, Bergen were examined

IV One hundred persistent nasal carriers of Staph aureus were selected

Staphylococcal *counts* from upper lip fingers and hands increased with increasing nasal counts In every case the nasal and skin strains were identical.

The majority of Staph. aureus on the *skin of nasal carriers are derived from the* nasal vestibule

The dispersal of Staph. aureus into the air on bed making also increased with increasing nasal counts but there was better correlation between skin (fingers and hands) and air counts than between nasal and air counts. Ninety-eight per cent of the strains in air samples were identical with the nasal and skin strains.

The *heaviest* dispersers of staphylococci among nasal carriers are those who yield the highest numbers of organisms on the skin (fingers and hands) Usually they also have the highest nasal counts.

Throat-carriers disperse far less organisms than nasal carriers.

V Two groups of 20 and 30 persistent nasal carriers of Staph. aureus were treated *with framycetin-gramicidin nasal spray 4* times *daily for 3 and 7 days respectively*

The staphylococcal nasal counts fell from high pre treatment values to less than 0 01 per cent of the original counts on the day after completion of treatment They remained relatively low for 4—7 days then rose during the following week to about the same *level* as *before* treatment

Forty persistent nasal carriers were treated with framycetin-gramicidin nasal spray for 3 days. The counts from nose upper lip fingers and hands fell from high pre-treatment values almost to nil the day after completion of therapy The same

applied to aerial dissemination on bed making

When the nasal counts increased after treatment, the skin and air counts also increased.

Framycetin-gramicidin nasal spray is a valuable measure for preventing staphylococcal dissemination from nasal carriers.

VI The numbers of staphylococci on various skin areas of 14 persistent perineal carriers and the degree of aerial dissemination on bed making increased with increasing perineal counts.

The heaviest dispersers among the perineal carriers dispersed far greater numbers of staphylococci into the air than the nasal carriers (chapter IV) Perineal carriers represent a greater problem in the control of hospital infection than their frequency would suggest.

Five perineal carriers were treated by washing their perineum and adjacent areas with hexachlorophane emulsion. The skin and air counts fell from high pre-treatment values practically to nil. Hexachlorophane skin disinfection is a valuable measure for controlling the staphylococcal dispersal from perineal carriers.

VII Self-contamination and aerial dispersal of Staph. aureus were investigated in 15 patients with various staphylococcal skin lesions.

Two patients with extensive pyodermias yielded far greater skin and air counts than individuals with minor skin infections. Patients with widespread staphylococcal infected skin lesions are among the heaviest dispersers.

Fourteen patients were treated by washing their skin lesions with hexachlorophane emulsion In the course of 2—11 days, all were converted from heavy dispersers almost to non-dispersers, and the lesions began to heal. Hexachlorophane skin disinfection is a valuable measure for preventing dispersal of Staph. aureus from patients with staphylococcal skin lesions.

Seven patients developed their lesions while in hospital. The strains causing the lesions were identical with those demonstrated in nose, throat or on the perineum before the lesions developed

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Appendix table *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers*
Estimated no. of bacteria (no. of colonies × dil. in thousands)

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
1	1	11,040	0.12	1 12	2.46	1 14	3.60	280.0	3.300
F	2	5,320	<0.04	0.56	<0.02	0.96	0.96	20.0	2.900
14	3	14,840	0.80	0.56	6.54	5.54	12.08	280.0	10 100
2	4	188	<0.04	<0.02	0.08	<0.02	0.08	<0.5	0.100
F	5	448	<0.04	0.16	0.24	<0.02	0.24	0.5	0.200
72	6	192	<0.04	<0.02	0.24	<0.02	0.24	<0.5	0.200
3	7	264	<0.04	0.28	0.28	0.18	0.46	0.5	0.200
M	8	120	<0.04	0.28	0.08	<0.02	0.08	<0.5	0.100
58	9	156	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
4	10	3,040	<0.04	<0.02	6.24	<0.02	6.24	60.0	0.500
F	11	4,240	<0.04	1 16	0.96	<0.02	0.96	40.0	0.800
24	12	4,600	<0.04	0 76	2.64	<0.02	2.64	40.0	0.800
5	13	40	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
M	14	28	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.050
53	15	12	<0.04	<0.02	0.06	<0.02	0.06	<0.5	0.025
6	16	104	0.04	<0.02	0.08	<0.02	0.08	<0.5	0.200
F	17	120	<0.04	0.12	<0.02	<0.02	<0.02	<0.5	0.100
33	18	728	2.00	0.08	0.48	<0.02	0 48	1.5	0.300
7	19	200	1.32	0.04	0.08	<0.02	0.08	3.5	0.500
F	20	260	49.60	<0.02	<0.02	<0.02	<0.02	0.5	0.800
14	21	212	12.32	0.68	0.24	0.56	0.80	14.0	1 400
8	22	4,120	0.12	0.96	3.26	0.10	3.36	28.0	1.600
M	23	1,240	<0.04	0.04	0.32	<0.02	0.32	42.0	2.800
17	24	4,640	<0.04	14 40	<0.02	8.08	8.08	22.0	5 100
9	25	8,640	<0.04	0.72	<0.02	0.64	0.64	100.0	0.900
M	26	2 480	0.04	8.82	0.64	0.64	1.28	60.0	1 400
48	27	2,080	<0.04	4.08	1 44	0.24	1.68	40.0	0.500
10	28	9,240	0.32	0.60	6.00	1 44	7 44	380.0	4.900
F	29	2,840	<0.04	0.04	1.08	0.08	1 16	100.0	2.900
60	30	3,240	1.20	0.16	0 48	0.16	0.64	100.0	4 100

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Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers*
Estimated no. of bacteria (no. of colonies × dil. in thousands)

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
21	61	4,328	<0.04	0.24	0.08	2.44	2.52	46.0	6.200
M	62	8,640	<0.04	12.72	0.68	3.04	3.72	78.0	3.800
16	63	8,200	<0.04	2.80	0.16	1.60	1.76	64.0	4.600
22	64	792	0.32	0.16	0.04	0.04	0.08	8.0	0.800
F	65	1,240	0.28	0.24	0.04	0.68	0.72	8.0	0.900
20	66	1,268	0.24	0.12	0.16	<0.02	0.16	12.0	1.400
23	67	174	<0.04	0.02	0.08	<0.02	0.08	14.0	0.200
M	68	124	0.04	0.04	0.24	<0.02	0.24	1.5	0.500
21	69	120	0.04	0.04	0.16	<0.02	0.16	0.5	0.200
24	70	12,960	<0.04	1.28	0.56	0.08	0.64	10.0	0.600
M	71	17,120	<0.04	17.52	0.40	2.00	2.40	28.0	0.400
65	72	9,440	<0.04	2.80	2.64	0.40	3.04	56.0	0.500
25	73	3,040	0.10	0.16	0.26	0.06	0.32	100.0	2.000
F	74	1,004	0.12	3.44	<0.02	0.08	0.08	40.0	1.000
48	75	1,620	0.88	1.88	0.90	0.86	1.76	16.0	1.300
26	76	800	0.04	0.52	0.22	0.06	0.28	6.5	2.000
F	77	152	<0.04	<0.02	0.04	<0.02	0.04	1.5	0.700
30	78	344	<0.04	0.04	0.30	0.02	0.32	12.0	0.500
27	79	18,840	0.56	9.28	101.00	22.00	123.00	480.0	21.400
M	80	18,120	<0.04	31.00	11.60	2.40	14.00	100.0	16.300
29	81	14,040	<0.04	6.00	5.00	1.00	6.00	400.0	10.400
28	82	80	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
F	83	92	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
24	84	112	0.04	0.56	0.20	<0.02	0.20	0.5	0.025
29	85	1,320	0.04	0.04	0.24	0.32	0.56	4.0	1.400
F	86	4,640	<0.04	0.08	0.18	0.14	0.32	20.0	1.200
70	87	680	0.04	0.04	1.04	<0.02	1.04	2.0	1.300
30	88	360	0.04	0.08	0.72	<0.02	0.72	4.0	0.200
F	89	160	<0.04	0.02	0.08	<0.02	0.08	2.0	0.200
33	90	992	<0.04	0.08	0.16	0.08	0.24	6.0	1.200

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers. Estimated no. of bacteria (no of colonies × dil in thousands)*

Pat. no., sex, age (yrs.)	Exp no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
11	31	16	0 08	<0 02	<0 02	<0 02	<0 02	<0.5	<0.025
M	32	28	<0 04	<0 02	<0 02	<0 02	<0 02	<0.5	<0.025
29	33	20	<0 04	<0 02	<0.02	<0 02	<0 02	<0.5	<0 025
12	34	1,264	<0.04	0 08	2.40	0 08	2 48	40.0	0.200
F	35	5,360	0 04	0 12	2 16	<0 02	2 16	20 0	0 400
47	36	8,800	<0 04	4 04	0 08	2 48	2.56	60 0	1.300
13	37	360	0.20	0.84	0 08	<0.02	0 08	<0.5	0.200
M	38	120	0.24	<0.02	0 16	0 16	0.32	1.0	0.200
16	39	120	<0 04	0 16	0 08	<0 02	0 08	<0.5	0 100
14	40	1,240	<0 04	0.32	0.24	<0 02	0.24	8.5	1 100
F	41	1 020	<0.04	0 1?	1.52	<0 02	1.52	13.5	1 400
43	42	4 400	<0 04	0 72	5.84	<0 02	5 84	32 0	3.200
15	43	1 120	<0 04	0.64	0.08	<0 02	0 08	2.0	1 400
M	44	440	<0.04	0 08	1 12	0 08	1.20	1.5	0.500
19	45	1 360	0.04	0 16	1.58	<0 02	1.58	6.5	1.200
16	46	3 480	0.24	0.12	12.84	0 64	13.48	40 0	3 600
F	47	2 880	0.96	3 76	21 68	0.24	21.92	40.0	4 100
64	48	5 680	0.52	0.24	2 08	<0 02	2 08	40 0	2 700
17	49	16,240	<0 04	0.20	2.88	0 16	3 04	10 0	11.500
F	50	14,240	<0 04	2 04	432 00	25 60	457.60	1,358.0	19 400
65	51	2?,560	0 08	1 24	20.80	0.32	21 12	1 120 0	14.600
18	52	40	<0 04	<0 02	<0 02	<0 02	<0 02	<0.5	<0.025
M	53	40	<0 04	<0 02	<0 02	<0 02	<0 02	<0.5	<0 025
16	54	20	<0 04	<0 02	<0 02	<0 02	<0.02	<0.5	<0 025
19	55	280	<0 04	<0 02	0 06	<0 02	0 06	0.5	0 100
M	56	464	<0 04	0 08	0 06	<0 02	0 06	1 5	0 100
42	57	320	<0.04	<0.02	0 10	<0 0?	0 10	0.5	0 100
20	58	2,400	<0 04	5.60	0.36	3.24	3 60	12.0	4.900
M	59	2 756	<0 04	3.36	2 08	0 14	2.20	6.5	3 700
52	60	1,280	<0.04	2 04	0.26	0 10	0.36	7.5	3.300

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil. in thousands)*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers Left	Fingers Right	Fingers Sum	Hands	Air contamination (bed making)
41	121	3,440	<0.04	0 40	2.24	<0.02	2.24	2.0	1.500
F	122	3,920	<0.04	0.12	9.52	0.60	10 12	12.0	1.900
73	123	2,800	<0.04	0.08	1 44	<0.02	1 44	4.0	1.200
42	124	97	0.08	0.16	0.02	0.02	0.04	4.0	0.300
M	125	208	<0.04	0 12	0 12	0.62	0.74	6.0	0 400
57	126	1 120	0.24	0.24	0.76	<0.02	0.76	2.0	0 400
43	127	9,200	0.16	1 40	5.00	0 12	5.12	60.0	[illegible]000
F	128	1 440	<0.04	0.88	4.80	0.96	5.76	12 0	1.200
66	129	2,400	<0.04	0.04	24.00	0.40	24 40	12.0	4.000
44	130	4,040	<0.04	0.68	1 76	1.64	3 40	56.0	2.800
M	131	880	0.32	1 46	0 12	0.08	0.20	12.0	1 100
28	132	1 120	<0.04	0 48	22.80	0.32	23 12	35.0	[illegible]400
45	133	2,080	<0.04	0.96	3.60	21.20	24.80	122.0	2.500
M	134	2,240	<0.04	0.24	0.36	1.04	1 40	60.0	3 700
24	135	880	<0.04	2.44	1.64	<0.02	1.64	32.0	2.200
46	136	2,040	<0.04	0.04	0.24	<0.02	0.24	4.0	0.300
M	137	1,800	<0.04	0.08	1.04	<0.02	1.04	20.0	0.900
24	138	4 120	<0.04	0.04	<0.02	1.00	1.00	60.0	0.800
47	139	240	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0 100
M	140	1,200	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
70	141	2,840	<0.04	<0.02	0 32	0.04	0.36	8.0	0.500
48	142	1,200	<0.04	0.12	1.32	0.32	1.64	28.0	6 400
F	143	8,240	<0.04	1.26	4 42	0.12	4.54	46.0	9.000
50	144	9,200	<0.04	1.92	1.60	0.04	1.64	10.0	3 100
49	145	2,800	2.04	4.00	11.00	0.88	11.88	52.0	0.700
M	146	5,440	0.20	6.00	0.92	0.60	1.52	71.0	1 100
15	147	480	0.32	1.20	0.04	0.56	0.60	10.0	0.900
50	148	2,480	<0.04	0.88	0 04	8.16	8.20	42.0	1.300
F	149	840	<0.04	2.84	0.32	<0.02	0.32	22.0	0.600
47	150	3,420	<0.04	0.16	0.32	<0.02	0.32	15.0	0.900

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil in thousands)*

Pat. no., sex, age (yrs.)	Exp. no	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
31	91	1,280	<0.04	4.80	1.04	<0.02	1.04	14.0	0.500
F	92	620	0.28	0.16	0.24	0.08	0.32	4.0	0.700
55	93	4,240	0.16	0.92	3.24	0.16	3.40	16.0	1.800
32	94	176	<0.04	0.08	<0.02	<0.02	<0.02	4.0	0.100
M	95	216	<0.04	0.04	0.08	<0.02	0.08	1.0	0.025
18	96	180	<0.04	0.12	0.28	0.04	0.32	2.0	0.100
33	97	400	0.72	0.04	0.08	<0.02	0.08	4.0	0.100
F	98	880	1.64	0.08	0.16	0.08	0.24	4.0	0.100
43	99	1 000	0.12	0.04	0.04	0.20	0.24	14.0	0.300
34	100	108	<0.04	0.04	0.48	<0.02	0.48	2.0	0.100
F	101	24	<0.04	<0.02	0.20	<0.02	0.20	<0.5	0.100
64	102	108	<0.04	<0.02	0.84	0.04	0.88	1.0	0.100
35	103	320	<0.04	0.08	<0.02	0.04	0.04	6.0	1.100
F	104	2 040	<0.04	2.04	1.44	2.20	3.64	100.0	4.900
15	105	320	<0.04	0.08	0.32	<0.02	0.32	2.0	2.400
36	106	2 920	0.16	2.44	2.40	2.48	4.88	172.0	0.700
F	107	1,600	0.68	2.88	0.28	<0.02	0.28	12.0	0.200
61	108	640	0.16	0.04	0.04	<0.02	0.04	2.0	0.400
37	109	2	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
F	110	1	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
17	111	3	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
38	112	12	<0.04	<0.02	0.02	<0.02	0.02	<0.5	0.100
F	113	28	<0.04	<0.02	0.16	<0.02	0.16	<0.5	0.100
41	114	48	<0.04	<0.02	0.02	<0.02	0.02	1.0	<0.025
39	115	2	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
M	116	1	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
45	117	1	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
40	118	32 080	3.28	25.60	56.00	12.40	68.40	720.0	39.800
M	119	22 520	1.20	41.00	24.00	0.48	24.48	360.0	14.400
52	120	14,200	1.24	37.00	4.08	26.00	30.08	820.0	13.200

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil. in thousands)*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
61	181	332	<0.04	0.46	0.14	0.18	0.32	19.5	1 400
M	182	640	<0.04	0.52	1.62	0.20	1.82	50.5	3.800
62	183	1 160	<0.04	0.60	0.48	0.04	0.52	23.0	1.600
62	184	364	<0.04	0.04	0.22	<0.02	0.22	3.5	0.100
M	185	64	<0.04	<0.02	0.16	<0.02	0.16	0.5	0.100
52	186	400	<0.04	0.94	1.06	0.02	1.08	0.5	0.600
63	187	880	<0.04	<0.02	<0.02	<0.02	<0.02	2.5	0.100
F	188	600	<0.04	<0.02	0.04	<0.02	0.04	<0.5	<0.025
51	189	920	<0.04	<0.02	0.06	<0.02	0.06	2.5	0.200
64	190	240	<0.04	0.08	0.22	0.12	0.34	<0.5	0.600
M	191	364	<0.04	0.08	0.08	<0.02	0.08	1.5	0.400
27	192	652	<0.04	0.82	0.64	<0.02	0.64	<0.5	0.300
65	193	20	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
M	194	60	<0.04	<0.02	<0.02	0.06	0.06	<0.5	0.050
59	195	28	<0.04	<0.02	0.02	<0.02	0.02	<0.5	<0.025
66	196	480	<0.04	0.14	0.86	0.94	1.80	31.0	0.900
F	197	1,640	<0.04	0.42	0.98	0.54	1.52	12.5	0.300
23	198	920	0.04	0.14	1.38	0.06	1.44	9.5	0.300
67	199	3,240	0.20	7.94	1.02	8.26	9.28	28.5	5 700
F	200	1,800	0.20	0.42	1.68	18.62	20.30	23.5	2 100
74	201	4,040	0.04	6.22	16.58	8.54	25.12	46.5	2 700
68	202	360	0.04	0.02	0.02	0.04	0.04	<0.5	0.025
M	203	280	0.04	0.04	0.02	<0.02	<0.02	<0.5	0.025
52	204	800	<0.04	0.02	0.14	0.02	0.14	<0.5	0.025
69	205	400	<0.04	0.02	0.66	0.08	0.74	1.5	1.500
F	206	840	<0.04	0.06	<0.02	3.42	3.42	1.5	1.300
73	207	1,560	<0.04	<0.02	0.46	<0.02	0.46	1.5	1 100
70	208	24	<0.04	<0.02	0.04	<0.02	0.04	<0.5	<0.025
M	209	120	<0.04	0.18	<0.02	0.54	0.54	1.0	0.200
48	210	20	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carri*
Estimated no of bacteria (no. of colonies × dil in thousands)

Pat. no., sex, age (yrs.)	Exp. no	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
51	151	16	<0.04	<0.02	0.02	<0.02	0.02	0.5	0.100
M	152	8	<0.04	<0.02	0.02	<0.02	0.02	<0.5	0.025
20	153	8	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
52	154	880	1.60	0.12	0.28	0.28	0.56	0.5	2.200
F	155	1 640	0.80	2.88	1.06	0.02	1.08	7.5	0.700
57	156	800	<0.04	0.08	0.14	<0.02	0.14	0.5	1.300
53	157	800	<0.04	0.24	0.36	<0.02	0.36	1.0	0.200
M	158	160	<0.04	0.16	0.16	0.08	0.24	3.0	0.300
73	159	120	<0.04	<0.02	0.20	<0.02	0.20	1.5	0.200
54	160	920	<0.04	1.32	8.12	2.62	10.74	11.0	1.100
F	161	120	<0.04	<0.02	0.20	0.02	0.22	3.0	0.700
73	162	400	<0.04	1.24	2.08	<0.02	2.08	12.0	0.800
55	163	640	<0.04	0.84	1.44	0.42	1.86	8.0	0.600
F	164	320	<0.04	0.18	3.80	<0.02	3.80	7.0	0.900
23	165	3 960	<0.04	7.42	3.48	1.68	5.16	50.0	1.000
56	166	80	<0.04	<0.02	0.12	<0.02	0.12	<0.5	0.300
M	167	16	<0.04	<0.02	0.02	<0.02	0.02	<0.5	0.050
57	168	80	<0.04	0.10	<0.02	0.24	0.24	1.0	0.300
57	169	40	0.88	<0.02	0.32	0.04	0.36	1.5	0.200
F	170	48	1.60	0.02	0.64	0.02	0.66	3.0	0.100
69	171	20	0.68	<0.02	0.02	<0.02	0.02	<0.5	0.100
58	172	2	0.04	<0.02	<0.02	<0.02	0.02	<0.5	0.025
M	173	5	<0.04	0.02	0.04	<0.02	0.04	<0.5	<0.025
67	174	7	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
59	175	160	0.04	1.04	4.22	<0.02	4.22	14.5	0.200
F	176	1 160	1.24	0.44	1.08	<0.02	1.08	2.5	0.300
72	177	360	<0.04	0.12	0.16	0.04	0.20	0.5	1.000
60	178	280	<0.04	0.22	0.48	<0.02	0.48	8.5	0.700
M	179	120	0.08	1.04	0.58	<0.02	0.58	0.5	0.500
31	180	240	<0.04	0.06	0.08	<0.02	0.08	0.5	0.600

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carriers*
Estimated no. of bacteria (no. of colonies × dil. in thousands)

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
81	241	80	<0.04	<0.02	0.08	0.66	0.74	2.5	0.300
F	242	40	<0.04	0.16	2.62	0.06	2.68	1.5	0.500
61	243	24	<0.04	<0.02	0.16	<0.02	0.16	<0.5	0.200
82	244	608	<0.04	0.48	3.00	3.00	6.00	7.0	1.000
M	245	448	<0.04	0.62	0.84	<0.02	0.84	1.5	1.200
45	246	600	<0.04	1.02	0.08	<0.02	0.08	<0.5	0.300
83	247	680	0.04	0.68	0.20	<0.02	0.20	1.0	0.075
F	248	2,800	<0.04	0.02	<0.02	<0.02	<0.02	<0.5	<0.025
64	249	160	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
84	250	520	<0.04	0.08	0.44	4.22	4.66	10.5	2.500
M	251	680	<0.04	0.22	2.46	7.22	9.68	16.5	2.900
33	252	3,240	<0.04	3.68	0.42	1.82	2.24	8.5	7.500
85	253	320	<0.04	<0.02	0.06	<0.02	0.06	<0.5	0.300
F	254	2,800	<0.04	0.04	0.10	<0.02	0.10	1.5	0.200
49	255	640	<0.04	<0.02	0.72	0.18	0.90	9.5	0.100
86	256	560	0.04	0.10	<0.02	0.92	0.92	28.5	2.000
M	257	640	<0.04	<0.02	0.54	0.26	0.80	11.5	2.400
42	258	280	0.12	0.06	0.04	0.06	0.10	3.5	1.000
87	259	2,080	<0.04	<0.02	1.14	<0.02	1.14	4.5	2.000
M	260	3,640	<0.04	2.70	6.66	0.24	6.90	83.0	1.700
57	261	2,880	<0.04	0.44	6.22	0.16	6.38	16.5	2.100
88	262	4	<0.04	<0.02	0.04	<0.02	0.04	<0.5	<0.025
M	263	28	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
49	264	4	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
89	265	4,800	2.48	1.06	61.00	2.00	63.00	34.5	7.800
F	266	5,040	1.24	0.86	39.00	3.00	42.00	71.0	7.500
16	267	2,480	0.84	0.42	7.00	<0.02	7.00	9.5	8.700
90	268	7,240	2.04	0.62	49.00	1.42	50.42	171.0	7.600
F	269	6,960	1.92	<0.02	1.62	6.24	7.86	36.0	10.000
17	270	1,040	1.78	0.02	36.00	0.14	36.14	35.5	7.900

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci 100 nasal carr*
Estimated no. of bacteria (no. of colonies × dil. in thousands)

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
71	211	2 400	<0.04	<0.02	0.06	0.62	0.68	2.5	1.50
F	212	12 440	0.04	0.92	10.22	0.12	10.34	65.5	5.60
72	213	3 120	<0.04	0.24	0.46	0.06	0.52	30.0	1.10
72	214	480	<0.04	<0.02	0.28	<0.02	0.28	2.5	0.70
M	215	3 600	<0.04	14.00	14.00	3.00	17.00	45.0	4.30
66	216	1 960	0.08	0.32	0.88	<0.02	0.88	3.5	1.20
73	217	360	<0.04	0.16	0.06	<0.02	0.06	<0.5	0.400
F	218	1 840	<0.04	0.36	8.84	<0.02	8.84	8.5	1.200
58	219	640	<0.04	<0.02	0.10	<0.02	0.10	1.0	0.200
74	220	680	<0.04	1.30	2.52	<0.02	2.52	2.5	0.400
M	221	1 080	<0.04	0.12	0.38	<0.02	0.38	11.5	0.200
40	222	2 800	0.04	0.42	26.00	0.06	26.06	63.0	0.600
75	223	680	<0.04	0.54	7.04	0.02	7.06	20.0	1.700
F	224	840	<0.04	0.04	0.74	0.12	0.86	22.5	2.600
45	225	4 080	<0.04	0.12	0.32	3.84	4.16	20.0	2.100
76	226	2 400	<0.04	1.62	121.00	0.48	121.48	190.0	6.300
F	227	2,800	<0.04	0.16	11.00	0.06	11.06	122.0	7.500
52	228	3,240	0.08	1.66	6.18	0.42	6.60	33.5	6.200
77	229	680	<0.04	0.06	0.12	<0.02	0.12	1.0	0.300
F	230	108	<0.04	<0.02	0.08	0.22	0.30	<0.5	0.100
41	231	292	<0.04	<0.02	0.08	<0.02	0.08	<0.5	0.100
78	232	920	0.04	0.02	<0.02	0.16	0.16	<0.5	0.025
F	233	480	<0.04	0.04	<0.02	0.12	0.12	<0.5	0.025
44	234	120	<0.04	0.10	0.04	<0.02	0.04	0.5	0.025
79	235	11,240	<0.04	16.00	69.00	10.00	79.00	89.0	29.400
M	236	14 400	<0.04	16.00	191.00	48.00	239.00	155.0	43.400
51	237	14,320	<0.04	4.00	48.00	4.00	52.00	51.5	1.900
80	238	5 080	0.04	1.04	16.66	9.62	26.28	186.0	7.200
F	239	4,200	<0.04	0.90	4.98	2.80	7.78	31.5	3.800
65	240	6 080	0.08	2.62	129.00	6.00	135.00	149.0	5.600

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INDIVIDUAL PLASMA PHOSPHOLIPIDS

with special reference to the changes in pregnancy

By

OLLE VIKRO

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INDIVIDUAL PLASMA PHOSPHOLIPIDS

with special reference to the changes in pregnancy

By
OLLE VIKROT

GÖTEBORG 1965

The present thesis is based on the following papers

I Quantitative Determination of Plasma Phospholipids in Pregnant and Non pregnant Women, with Special Reference to Lysolecithin
Acta Med. Scand. *175* 443 1964

II Plasma Lipid Fractions, Including Individual Phospholipids, at Various Stages of Pregnancy
(together with A. Svanborg)
Acta Med. Scand. in press

III Plasma Lipids during the First Week after Delivery
(together with A. Svanborg)
Acta Med. Scand. in press

IV Individual Plasma Phospholipids in Healthy Young Women
(together with A. M. Högdahl)
Acta Med. Scand. in press

V Plasma Cholesterol Esterification and Lysolecithin Production In Vitro during Pregnancy Hyperlipemia.
(together with A. Svanborg)
Acta Med. Scand. in press

VI Plasma Lipids, Including Individual Phospholipids, in Pregnant Rats.
Acta Med. Scand. in press.

In the following these publications are referred to under their Roman numerals.

CONTENTS

INTRODUCTION

The phospholipids are important components of tissue structures and are also important building-blocks for the plasma lipoproteins (44). Changes in the phospholipid composition in the blood or in other tissues must, therefore, be of biological significance.

According to the "filtration" theory of atherogenesis, alterations in the plasma lipids have been considered to be of importance and a large number of studies have been performed on the blood lipids in different groups of individuals. In many such studies total phospholipid in plasma or serum have been determined, but so far only few investigations have dealt with individual phospholipid fractions.

The aim of the present investigations was to obtain additional information on the individual plasma phospholipids and the mechanisms influencing their levels.

At the start of this study in 1958 no method was available for the accurate determination of individual phospholipids from small blood samples, so that the first step was to develop such a method (I).

The method was used in various disorders with derangements of lipid metabolism and it was found that, in general, the phospholipid composition was rather constant but that pronounced changes occurred in the hyperlipemia of pregnancy. It was considered of interest to study these changes in more detail, since they could be expected to be due to the alterations in the hormonal balance. Observations in recent decades have demonstrated the important influence of many hormones in the regulation of the lipid metabolism [for reviews see Cook (19) and Marshall (68)]. Because of the sex difference in the morbidity of atherosclerosis, hormonal actions on lipid metabolism have been presumed to be of importance in the causation and also the treatment of atherosclerosis.

Studies were performed on plasma phospholipids at various stages of pregnancy (II) and during the first week after delivery (III). In paper IV the relation between the phospholipid composition and levels of other lipid fractions was studied in non-pregnant women in the fertile age. In paper V an experiment was performed on the activity in pregnancy of the plasma fatty acid transferase process, which has been presumed to participate in the formation of lysolecithin. Finally investigations were carried out to determine if alterations similar to those seen in humans, occur in the pregnant rat (VI).

NOTE ON NOMENCLATURE

The phospholipid fraction in plasma is inhomogeneous. The common feature which distinguishes phospholipids (also called phosphatides) from other lipids is that they contain one phosphoric acid residue. No generally accepted classification and nomenclature exists (2).

Most phospholipids contain glycerol and this group is sometimes called glycerophospholipids. To the glycerol residue is linked one or two fatty acids and to the phosphoric acid residue choline, ethanolamine or inositol. The glycerophospholipids which contain choline and two fatty acids were called *lecithin* in these papers. Most of the fatty acids are bound with an ester bond but a small proportion is bound with a vinylether linkage (plasmalogens) or with an ether linkage. The phospholipids which contain choline but only one fatty acid were called *lysolecithin*. The compounds containing ethanolamine instead of choline were called *phosphatidylethanolamine* (with two fatty acids) or *lysophosphatidylethanolamine* (with one fatty acid). Phosphatidylethanolamine is partly in the form of plasmalogens. The term "cephalin" has not been used as it has commonly been applied to various lipid fractions, even though it has sometimes been used synonymously with phosphatidylethanolamine. The compounds which *contain inositol linked to the phosphoric acid residue were called phosphatidylinositol*.

The non-glycerol-containing phospholipids in which the main longchain base is sphingosine, are sometimes called sphingophospholipids. The term used here was *sphingomyelin*. They also contain choline and one fatty acid, linked through an amide bond.

In these papers lipid levels have generally been given in molar concentration (or in micrograms lipid phosphorus in paper I). In many reports phospholipids are given in mg per 100 ml. Such values depend on the assumption of a mean molecular weight for phospholipids of 775 which has been found to be relevant for the phospholipids of normal persons (48) but which may not be applicable when the phospholipid composition changes.

PRESENT STUDY

Paper I describes a method which allows separation and quantitative determination of the four main plasma phospholipid fractions. After extraction of the lipids in plasma the phospholipids are separated by thin-layer chromatography on silica gel plates. The phospholipid spots are scraped off and the phosphorus content determined. As each molecule of phospholipid contains one phosphorus atom, this determination gives a measure of the percentage molar composition of the phospholipid fraction. Multiplication by the total amount of lipid phosphorus then gives the absolute levels. Small amounts of lysophosphatidylethanolamine and probably phosphatidylinositol were found but the concentration was too low to be measured accurately. During the testing of the method it was found that it is necessary to perform an immediate extraction of plasma, as lysolecithin is formed rather rapidly in vitro, even at room temperature. Finally preliminary results of the findings in pregnant women were given.

In paper II the findings in the hyperlipemia of pregnancy were confirmed and extended. Twenty-one women were studied once each at various stages of pregnancy and two women were studied with serial determinations. In addition to total and individual phospholipids, triglycerides and cholesterol were also determined. It was found that all lipid fractions increased progressively during pregnancy except lysolecithin, which decreased. Sphingomyelin increased less than total phospholipids so that there was a decrease in the percentage of sphingomyelin. Statistical calculations showed a correlation between the phospholipid composition and the total phospholipid level. Total phospholipids and cholesterol were also correlated to each other and the regression of cholesterol on phospholipids did not differ from that in non-pregnant women. The relation of triglycerides to the two other main fractions seemed to differ from that in non-pregnant women, with a more pronounced increase than of the other fractions. Some possible mechanisms for the hyperlipemia were discussed with special reference to what might cause the change in the phospholipid composition.

In paper III six women were followed with repeated determinations from the day of delivery and during the first puerperal week. The levels at delivery did not differ from those calculated for the end of pregnancy according to regression equations in paper II, which indicates that the alterations in the plasma lipids progress even near the end of the pregnancy and up to delivery. As early as the first postpartum day the

levels had changed in the direction towards non pregnant levels. During the following days further changes occurred but the women had not reached non pregnant levels at the time of the last blood samples.

Paper IV describes the results of the investigation of 23 healthy non pregnant women in the fertile age. The values for total phospholipids, cholesterol and triglycerides were in agreement with those from other studies from the same area of Sweden (65 105) The phospholipid composition was also similar to that found in other studies, except that the lysolecithin percentage was lower presumably because of the precautions taken to prevent lysolecithin formation in vitro (I) As in previous studies (17 31) the distribution of triglycerides was asymmetrical and became more symmetrical after the conversion to the logarithm of the triglyceride values In the statistical calculations the logarithmic values were used Triglycerides did not correlate with total phospholipids or cholesterol A positive correlation was obtained between total phospholipids and cholesterol The percentages of individual phospholipids were not correlated with the levels of total phospholipids, cholesterol or log triglycerides which indicates that the phospholipid composition is independent of the levels of these three fractions. The inter individual variations were comparatively small so that the phospholipid composition could be regarded as rather constant.

As it has been suggested (36) that lysolecithin in plasma may be partly produced by a transesterification process, observations were carried out to test the hypothesis that the low level of lysolecithin in pregnancy might be due to a lowered activity of this process (V) Plasma from 5 pregnant women and from 5 controls was incubated and the changes in free cholesterol lecithin and lysolecithin were determined. No difference was obtained between the two groups, and it was concluded that changes in the activity of the transesterification process probably do not cause the decrease of lysolecithin in pregnancy

In rats the phospholipid composition differs considerably from that in humans with a much higher percentage of lysolecithin and lower percentage of sphingomyelin. It was, however found that changes in phospholipid composition in pregnant rats were similar to those in human pregnancy (VI) It was suggested that the rat might be a suitable animal for experiments on the genesis of the hyperlipemia of pregnancy

DISCUSSION

In each paper the main findings have been discussed and in the following only some points will be considered.

Lipids are insoluble in water and are transported in plasma as lipoprotein complexes (44) The binding of the lipids to the protein moiety is not well understood but it is generally considered that the polar phospholipid molecules play an important role. Individual phospholipids have different physico-chemical properties and a closer knowledge of the plasma phospholipid composition must be of value for the understanding of lipid transport in blood.

Methods for determination of individual phospholipids

Some earlier methods for the determination of individual phospholipids were based on differential solubility [see Wittcoff (111)] and are now known to be gravely in error Partial hydrolysis methods with determination of hydrolysis products [e.g. that of Dawson (23)] have given much more reliable values but it was only with the advent of chromatographic methods that it was also possible to measure lysolecithin accurately Chromatography on silicic acid columns was first used (84) but it is difficult to get adequate separations with this method. Non-impregnated paper can separate phospholipids partly (4) but impregnation with e.g. silicic acid (66) gives much better separations. The capacity of the papers is rather low and accurate determination of the minor phospholipids is difficult (75).

At the start of this investigation attempts were made to improve the separation on silicic acid columns by varying the eluants. The silicic acid-silicate column of Rouser et al. (96) was also tried but overlapping between fractions was always evident. Fractions from columns could, to a certain extent, be further separated by paper chromatography but the method then became very laborious and few useful results were obtained.

With thin-layer chromatography it was easy to obtain excellent separations of phospholipids and the report of Habermann et al. (45) showed that, in addition, quantitative determinations could be made. It was therefore decided to develop this method further and to test its reliability The method described in paper I seems well suited for this purpose. It is reasonably accurate and needs only a small amount of plasma. While elution of spots might have increased the precision of the method (102) the direct determination of phosphorus is timesaving and thus allows a larger number of replicates to be made.

Previous studies on the plasma phospholipid pattern

In some studies changes in the plasma phospholipid composition have been noted in various conditions. Petersen (82 83) Phillips (87) and Gjone & Mendeloff (35) found changes in cases of liver disease with relative increase of lecithin and relative decrease of sphingomyelin Especially in biliary cirrhosis a low percentage of lysolecithin was obtained. Gjone & Mendeloff (35) also found high lysolecithin values in acute pancreatitis. Marinetti et al. (67) reported a low percentage of lysolecithin in some cases of myocardial infarction Nye & Waterhouse (76) obtained a higher proportion of sphingomyelin and lysolecithin and a lower proportion of lecithin in most but not all cases of the nephrotic syndrome. Christian et al (18) reported a family with a hereditary hyperlipidemia in whom the percentage of sphingomyelin tended to be low They also reported one child with familial hypercholesterolemia who had a low percentage of lysolecithin. Nothman & Proger (74) used a non-chromatographic method and reported increased percentages of phosphatidylethanolamine and phosphatidylserine in persons with atherosclerotic heart disease. The speci

Sex	Age	Diagnosis	Total phospholipids mM	% of total phospholipids PE	Lec	Sph	LL
F	31	Acromegaly in active phase	2.54	2.8	65.3	24.7	7.2
F	36	Cushing's disease	3.03	3.9	69.3	20.3	6.5
F	42	Diabetes	3.19	2.8	75.3	15.4	6.6
M	47	Diabetes	3.02	3.5	69.6	18.4	8.6
M	34	Diabetes	3.18	5.1	71.6	16.9	6.4
M	35	Diabetes	3.17	3.0	70.8	20.5	5.6
F	69	Diabetes	3.33	3.8	67.8	23.6	4.9
M	80	Diabetes	4.15	2.6	66.9	26.7	3.8
F	40	Hereditary hypercholesterolemia	4.29	2.8	65.7	25.1	6.4
M	49	Hereditary hypercholesterolemia	3.96	3.0	65.7	24.8	6.5
M	61	"Essential" hyperlipemia	5.04	3.8	69.0	22.5	4.7
F	39	Nephrotic syndrome	8.01	2.9	67.0	25.0	5.1
		Normal values	3.21	2.8	69.5	22.2	5.5
		S.D	0.38	0.4	1.4	1.5	0.9

Table I Plasma phospholipid composition in 12 patients with various diagnoses.
F = female. M = male. PE = phosphatidylethanolamine. Lec = lecithin.
Sph = sphingomyelin. LL = lysolecithin
S.D = standard deviation. *Normal values are those in 23 healthy women (IV).*

ficity of the method is doubtful and the results could not be confirmed by Wagener et al. (107) who, using thin-layer chromatography found no difference between "healthy" and atherosclerotic individuals. With the method described in paper I some observations have been made in diseases in which lipid metabolism is known to be affected and some findings are presented in table I. The results are similar to those found in healthy young women. No extensive control material has been collected from males or older women but from data in the literature no pronounced differences seem to exist between the sexes or between different age groups.

Individual phospholipids in hyperlipemia of pregnancy

The changes in pregnancy are, however, very distinct and must be considered as characteristic, even if not pathognomonic, of the hyperlipemia of pregnancy. It has been known for a long time that plasma lipids increase in pregnancy [for reviews of earlier work see Boyd (10) and Oliver & Boyd (78)] but determinations of individual phospholipids have not been made. In every case studied during the latter part of pregnancy obvious deviations from the normal phospholipid pattern have been observed. Most striking is the absolute and relative decrease of lysolecithin but there is also a relative decrease of sphingomyelin. These findings are not easily explained in terms of simple increases or decreases in lipoprotein fractions. Lysolecithin is found in all lipoprotein fractions which have been examined so far (29, 37, 85, 112) but most of the plasma lysolecithin is found in high-density (> 1.063) or very-high-density (> 1.21) lipoproteins (29, 37, 85, 86). While lipoprotein determinations in pregnant women have shown a relative decrease of high-density lipoproteins (38) or their counterpart as found by Cohn fractionation (97), electrophoresis (104) or column chromatography (20), the absolute amount has been found unchanged or increased. The decreased percentage of sphingomyelin also cannot be explained by the known shifts in lipoprotein composition. The preponderant increase in pregnancy is in lipoproteins of lower density than 1.063 and these have a higher sphingomyelin content than that found in whole serum (85).

No studies have been published on the phospholipid composition within lipoprotein fractions during pregnancy. It is possible that the phospholipid pattern within these fractions is changed during pregnancy. It may be noted that Cramér et al. (20) found an increased content of triglycerides in high-density lipoproteins during pregnancy. On the other hand, high-density and low-density lipoproteins are not homogeneous fractions but can be subdivided in many smaller fractions whose phospholipid composition has not been thoroughly investigated. It is therefore also possible that one or

more of these subfractions are produced in altered amount during pregnancy. An alternative explanation is the appearance, during pregnancy of completely new lipoproteins which has been suggested by Rejnek et al. (92).

Etiological factors

The mechanism of the *hyperlipemia* of pregnancy has not been clarified. In 1934 Boyd (10) listed 17 possible etiologies and many have since been added. It is evident that the presence of the fetus and the placenta must be responsible basically for the changes in the lipid metabolism. The physiologic processes are thoroughly changed during pregnancy and many mechanisms may therefore influence the lipid metabolism. But it is not surprising that the very pronounced changes in hormone production during gestation have been especially suspected as the causative factor.

Hormones. The endocrine changes during pregnancy are very complex. A new organ appears, the placenta, which produces large amounts of several hormones and possibly also interferes with the metabolism of other hormones (27). The fetus plays an important role in the metabolism of some hormones (25). *Changes have been described in several* of the maternal endocrine glands (51). The metabolic effects of several hormones are not well known and even less is known about the action of hormones in combination. It is therefore impossible to get a complete picture of what these endocrine changes may signify for the genesis of the hyperlipemia of pregnancy. No attempt will be made to *cover in full this relationship but some* points seem worth discussing.

The estrogens are formed in very large amounts in the feto-placental unit (26). The main objection against considering them as a possible cause for the hyper*lipemia has been that estrogens in several* studies have been shown to decrease plasma cholesterol, especially in the beta lipoprotein fraction, which is the opposite of the findings in pregnancy (78). This may not be a valid objection as most such studies have been performed on hypercholesterolemic persons, often men (19, 68). In fact, Berezin & v. Studnitz (5) showed an increase in cholesterol after estrogen treatment of women with normal *levels before* the treatment.

Furthermore different estrogenic compounds may have different actions. Estriol is *found in blood and* urine in especially large amounts during pregnancy. It is noteworthy that while most hormones return to normal levels in blood or urine in the first days after delivery, there is a slower decrease of the excretion of estriol with normal amounts at first after 3—4 weeks (26). The effect of estriol on lipid metabolism in humans seems *to have been studied in* only one investigation (80) in which it was *reported to be* qualitatively similar

to that of other estrogenic substances. This was, however a study in hypercholesterolemic men, and additional investigations might be of interest.

Another objection to estrogens as a lipemia-producing factor has been that after menopause, spontaneous or surgically induced, an increase in blood lipids has been reported (77 81 94) The menopause is, however not simply a withdrawal of estrogens, and the mechanism of changes in lipid metabolism in the climacterium remains to be established.

The importance of dosage for the relative effects of estrogenic hormones on different lipids must also be taken into consideration (89). The pregnant woman is exposed to very large amounts of estrogens for a long time, an effect which cannot easily be reproduced experimentally It is, however of interest that in studies in man in which individual phospholipids have been analysed after estrogen treatment (47 54) the changes in the phospholipid patterns have been in the direction of pregnant values. Svanborg & Vikrot (unpublished) gave altogether 160 mg estradiol benzoate (Follicyclin "Ciba") i.m. during 2—3 weeks to three ovariectomized women. Lysolecithin decreased from about 6 to about 3.5 per cent of the total phospholipids.

Progesterone is also produced in huge amounts during pregnancy (51). Its effects on lipid metabolism are largely unknown. Some studies in humans with small doses for short times have shown no significant changes in cholesterol or phospholipids (79 80) However in dogs Zanetti & Tennent (113) found cholesterol to increase after large doses. Preliminary studies have shown only insignificant changes in the phospholipid pattern after progesterone given to three ovariectomized women (500 mg Proluton-Depot "Schering" daily for 10 days), (Svanborg & Vikrot, unpublished)

Other hormones produced in the hypophysis, in the adrenals and in the thyroid gland are known to influence the synthesis, the oxidation and the mobilization of lipids as well as the plasma lipid level Alterations in the production and metabolism of these hormones occur during pregnancy (51) At the present state of knowledge it is not possible to explain the hyperlipemia of pregnancy from changes in these endocrine functions. The data presented above with normal phospholipid pattern in one patient with active acromegaly and one with Cushing's disease hints that the growth hormone or adrenal steroids are at least not the main factors responsible for the phospholipid changes.

The chorion gonadotropin is a hormone specific for pregnancy Even though it has been used in the treatment of obesity there seem to be no experimental investigations and its effect on lipid metabolism is not known (39) It is produced in large amounts early in pregnancy (51) when blood lipids only have started to increase and it seems unlikely that it is of major importance as a cause of the hyperlipemia.

Recently another protein hormone produced in the placenta has been de-

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called fatty acid transferase (36, 37) seems to be formed in the liver (14). The physiological importance of this plasma enzyme activity is not known. It transfers one fatty acid from lecithin to cholesterol and thus produces lysolecithin and cholesterol esters. The findings in paper V are evidence against a lowered activity of this enzymatic process as the cause of the lower level of lysolecithin in pregnancy.

Placenta. The metabolism of lipids within the placenta has not been thoroughly studied and it is not known to what extent, if any, it may contribute to the changes in blood lipids. The findings in paper III that in the puerperium there is not a rapid return of plasma lipids to non-pregnant levels, suggest that the direct placental metabolism of lipids is not the main cause of the hyperlipemia.

Fetus. The lipid metabolism of the fetus itself has generally been assumed to be "autonomous" and it is thought that the fetus produces its lipids from non-lipid sources mainly (46), even if some data speak for a transport of non-esterified fatty acids across the placenta (106). Boyd & Wilson (13) suggested that the child takes up phospholipids and cholesterol from the placenta. Data from a study of Cramér & Vikrot (21) show a non-pregnant phospholipid pattern in cord blood and are evidence that phospholipids are not transported by passive diffusion across the placental membrane. Whether an active transport of lipids occurs has not been studied adequately in humans. In any case, a transfer of lipid to the fetus would tend to decrease plasma lipids. A transfer of lipids from the fetus to the mother seems unlikely.

Other factors. Other factors which may be of importance for the mechanism of the hyperlipemia of pregnancy are e.g. nutritional factors which have been discussed in paper II.

Hypothesis. It is probable that the mechanism for the hyperlipemia is a complex one and that many factors contribute to its production. From the findings in the present investigation no definite conclusion as to the genesis of the hyperlipemia can be drawn. It seems, however, a reasonable working hypothesis that one important factor especially for the change in the phospholipid pattern, is an effect of estrogens, presumably mediated via the liver.

Effects of the hyperlipemia of pregnancy

Immediate effects. The effects of the hyperlipemia are even less well understood than the etiology. It is tempting to seek a physiological meaning in the drastic change in lipid homeostasis but no really convincing explanation has been given.

From a teleological point of view it has e.g. been suggested that the hyperlipemia is involved in the increased

scribed (34 43 52 55 56 58) It has immunological properties similar to human growth hormone and has prolactin like and growth-hormone-like activity Its blood levels increase right up to delivery (59) Important metabolic effects have been assumed for this hormone (58) though this has not yet been thoroughly investigated. Friesen (34) did not, however find any lipolytic properties but increased conversion of glucose to fat in investigations on epididymal fat pads. On the other hand Bleicher et al (6) in a short communication described a lipolytic substance in the placenta and in the sera of pregnant women They suggested that this substance might be related to the growth hormone-like substance discussed above. Further investigations in this area will be of great interest.

The change in the carbohydrate metabolism in pregnancy has been reviewed by Burt (16) who concludes that pregnancy is diabetogenic and that the essential change is that of insulin antagonism It was suggested (22) that the impaired carbohydrate metabolism might be responsible for the hyperlipemia of pregnancy The findings in patients with diabetes mellitus (see table) show that the alterations in metabolism and in hormonal balance in this condition do not influence the phospholipid composition in plasma Nevertheless, it is possible that changes in carbohydrate metabolism may explain part of the hyperlipemia, e.g the increase in free fatty acids (7 15 61 73) and triglycerides. On the other hand, according to the concepts presented by Randle et al. (91) the altered carbohydrate metabolism may be secondary to the elevation of free fatty acids and the tendency to ketosis which has been described in several earlier studies (8 9 40 42 95 99) Both free fatty acids and ketone bodies tend to inhibit glucose uptake and metabolism. However other anti insulin factors are probably also of importance e.g insulin degradation in the placenta (33)

Liver The changes in blood lipids during pregnancy are similar to those found in some cases of liver disease, especially biliary cirrhosis (35 87) Low levels of lysolecithin have been observed in instances of obstructive jaundice (Svanborg & Vikrot, unpublished) Some findings which speak for a change in liver function during pregnancy have been discussed in paper II It is possible that the hepatic involvement is due to hormonal effects e.g to estrogens. High doses of estradiol and estriol have been shown to cause alterations in liver function (72) and synthetic steroids have often produced icterus [for review see Adlercreutz (1)] The mechanism of the hyperlipemia in liver diseases is not well understood (87) but further studies on the lipid metabolism of the liver in pregnancy are indicated The findings of similar changes in blood lipids in the pregnant rat (VI) as in humans, would make this a suitable experimental animal.

In this context it may be mentioned that the enzyme system which has been

any important differences between normal pregnancy and preeclampsia in the case of plasma phospholipid changes.

Long-term effects Long term effects of the hyperlipemia may also be considered. In some studies (e.g. 32, 90) a higher incidence of diabetes mellitus in multiparae has been found. Though other factors cannot be excluded, e.g. higher fertility in women genetically disposed to diabetes (53 69) one mechanism might be the repeated bouts of hyperlipemia. As previously mentioned this may inhibit the glucose utilization and may thus ultimately "wear out" the pancreatic islets in susceptible persons.

Studies on the incidence of atherosclerosis and its complications in various groups are difficult to evaluate. However it seems well established that coronary heart disease is much less common in fertile women than in men of the same age (57) Myocardial infarction during pregnancy is an extreme rarity in spite of the increased lipid levels (108) Winkelstein et al. (109 110) have studied women with coronary artery disease and found a higher frequency of total pregnancies than in control materials. However this was due to a higher frequency of abortions while the number of live births did not differ Most abortions occur early in pregnancy when blood lipids have only begun to increase and therefore these studies suggest that pregnancy is not an atherogenetic factor in spite of the repeated exposure of the vessel walls to high lipid levels. If this is confirmed the explanation may be that the time of exposure is too short. But it is also possible that hyperlipemias of different etiology may not be atherogenetic to the same degree. Perhaps the altered phospholipid composition during pregnancy is a factor which tends to prevent atherosclerosis. Coronary atherosclerosis has been reported to be rare in biliary cirrhosis (100) in spite of the high lipid levels in this condition. The phospholipid composition in biliary cirrhosis is similar to that found in pregnancy (35 87).

production of steroid hormones (51) but most tissues seem able to synthesize the parent compound of the steroid hormones, cholesterol from low-molecular precursors (41)

Another common proposition is that the hyperlipemia *is a preparation for* lactation One piece of strong evidence against this is the finding that, in the cow plasma lipids decreased during pregnancy and increased after parturition (28 101)

One might also speculate whether the hyperlipemia might be of some importance for protection against blood loss at delivery as there is some evidence that lipids favor blood coagulation and inhibit fibrinolysis (50 70 71 88)

The most plausible teleological explanation would seem that the hyperlipemia is an adaptation for the needs of the fetus. As already discussed it is not likely that the fetus can utilize directly the maternal blood lipids but according to the concepts of Bleicher et al. (6 7) the hyperlipemia might allow the fetus to utilize a larger proportion of glucose and gluconeogenic precursors. It is known that the tissues utilize free fatty acids in proportion to the blood level (3) and according to the hypothesis of Randle et al (91) the high level of fatty acids and tendency to ketosis would reduce the uptake of glucose in the maternal tissues and save it for the fetus. However such a hypothesis does not explain the change in the pattern of the phospholipids which are not considered to be used for nutritive purposes.

Luukkainen & Csapo (64) suggested that *phospholipids might be involved in* the initiation of labor In such case one might expect changes in the plasma phospholipid concentration or composition just before or at delivery As this *does not occur (111) plasma phospho*lipids can hardly be responsible for the initiation of labor

The physico-chemical state of the blood may be influenced by the phospholipid changes, both quantitative and qualitative. It is known that phospholipids are strongly surface-active substances and it has e.g been found that the physical properties of a mixed lecithin lysolecithin sol are dependent on the relative proportions of each component (93) Some earlier investigations have been made on the physical properties of the blood in pregnancy A lowering of the surface tension was reported using various methods (30 62, 63 98 103) While the viscosity of the blood decreased in pregnancy this seemed to depend on the reduced hematocrit and the viscosity of the plasma *was not* changed (49) Too little is known of the way phospholipids are transported in the blood to allow any conclusions on the influence they may have on these physical properties. Further investigations are needed

Changes in lipid metabolism during pregnancy have sometimes been considered responsible for pregnancy complications, *such as toxemia* (11 12) No clearcut findings have been obtained (24 60) Preliminary observations (Spetz & Vikrot, unpublished) have not shown

any important differences between normal pregnancy and preeclampsia in the case of plasma phospholipid changes.

Long term effects. Long-term effects of the hyperlipemia may also be considered. In some studies (e.g. 32, 90) a higher incidence of diabetes mellitus in multiparae has been found. Though other factors cannot be excluded, e.g. higher fertility in women genetically disposed to diabetes (53 69), one mechanism might be the repeated bouts of hyperlipemia. As previously mentioned this may inhibit the glucose utilization and may thus ultimately "wea out" the pancreatic islets in susceptible persons.

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SUMMARY

A method has been described for the quantitative determination of phosphatidylethanolamine, lecithin, sphingomyelin *and lysolecithin in extracts from 1* ml of plasma

During studies of the individual plasma phospholipids in various diseases with derangements of lipid metabolism it was found that the plasma phospholipid composition was rather constant in most cases but that pronounced changes occurred during pregnancy

Twenty-one women were studied once each at various stages of pregnancy and two women with serial determinations A continuous increase of all lipid fractions occurred with the duration of pregnancy with the exception of lysolecithin which decreased Sphingomyelin increased less than the total phospholipid fraction

Six women were studied on the day of delivery and repeatedly during the first puerperal week A distinct change in the direction towards non pregnant levels was observed as early as the first postpartum day but non pregnant values had not been reached at the end of the period

In twenty three healthy non-pregnant women in the fertile age the phospholipid composition was essentially constant and was not related to the levels of total phospholipids, cholesterol or triglycerides.

In vitro studies suggested that the lysolecithin decrease in pregnancy was not due to a lowered activity of the *plasma fatty acid transferase activity* which has been thought by some authors to be of importance for the lysolecithin level in plasma.

In pregnant rats similar results were obtained as in humans

Several factors which might cause the hyperlipemia of pregnancy were discussed It was assumed that one important factor especially for the change in the phospholipid pattern, is an estrogen action, presumably mediated via the liver

Possible effects of the hyperlipemia were discussed

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SUPPLEMENTUM 434

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By

A. F. Lever

ACCOMPANIES VOL. 178

STOCKHOLM 1965

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CONTENTS

INTRODUCTION

In 1942, Kuhn and Ryffel (1) first suggested that urine was concentrated by countercurrent multiplication in the renal medulla. Subsequently they and others further suggested that the multiplication occurred within the loop of Henle, that the renal medulla became hypertonic as a result and that this in turn led to the concentration of urine by reabsorption of water from the collecting duct. Since that time, both the hypertonicity (hyperosmolality) of the renal medulla during dehydration (2, 4 6) and the reabsorption of water from the collecting duct (7–9) have been confirmed experimentally The mechanism by which the initial multiplication produces the hypertonicity however has remained less clear Most of the hypotheses proposed have suggested that the multiplication process occurred either within (5 10–12) or in combination with (13–14) the loop of Henle.

The present paper is concerned with an alternative possibility that the multiplication occurs in the bundles of looped blood vessels (the *vasa recta*) within the renal medulla, and that the loop of Henle is not directly involved in this process. The possibility that the vasa recta might be capable of such multiplication has been discussed previously by others (17 18, 22, 75) but has not, as far as I am aware, been considered as the primary event in the absence of contribution by the loop of Henle.

These possibilities are considered in three sections. The first is concerned with evidence that multiplication occurs within the vasa recta, the second with the excretion of urea by the loop of Henle and the third with a subsidiary hypothesis of urine dilution.

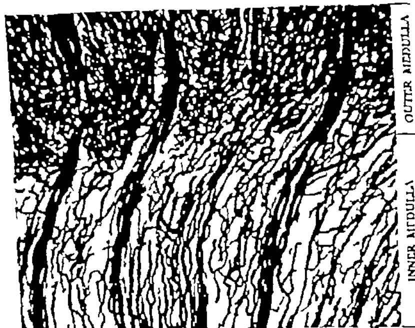


Fig. 2. Blood vessels at the junction of inner and outer medulla in the rat. Note (1) The marked change in the form of the capillary plexus at the junction of inner and outer medulla. (2) The distinction between bundle and plexus in the outer medulla.

From Moffat and Fourman (29) reprinted by permission of the *Journal of Anatomy*

as a result of a difference in hydrostatic pressure combined with a small difference in the permeability of the vessels to sodium and water. Fluid with a raised concentration of sodium descends to the tip of the loop and rises in the ascending limb (Fig. 1B) where the increase in molality permits further transverse water movement, thereby initiating a progressive sequence of multiplication. Sodium would tend to move in the opposite direction to water in this process and might, therefore, become sequestered in the medulla. In contrast to the sodium pump hypothesis, the source of energy in the present theory is hydrostatic pressure.

In the following sections it is proposed to consider this theory in relation to the anatomical and physiological data on urine concentration.

Anatomy of the Vasa Recta

Moffat and Fourman (29 and 30) distinguish two relatively independent vascular systems within the renal medulla: the vasa recta bundle and a capillary plexus (Figs. 2 and 3). In the present theory it is suggested that multiplication occurs within the bundle, and that the capillary plexus is in part responsible for the dilution of tubular fluid (*see* Part III).

(A) *Vasa recta bundle.* – The efferent arterioles of juxtamedullary glomeruli enter the renal medulla and divide to

PART I

THE VASA RECTA AND URINE CONCENTRATION

Countercurrent Multiplication

In principle, countercurrent multiplication depends upon two factors the movement of fluid in a looped tube and the presence of some active process capable of maintaining a difference in concentration between the closely apposed limbs of the loop This principle is equally applicable to the sodium pump hypothesis described below and to the present suggestion that multiplication is the result of ultrafiltration in the vasa recta.

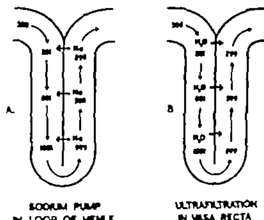


Fig 1 Two hypothetical processes of countercurrent multiplication

A. Sodium is actively pumped from ascending limb of loop of Henle, thereby establishing a small gradient of osmolality between the limbs. Hyperosmololar fluid rises in ascending limb. This permits further transverse movement of sodium and initiates multiplication. Numbers refer to hypothetical osmolality (mOsm/kg)

B. Similar process occurs with ultrafiltration of water from descending to ascending limb.

(A) *Sodium pump hypothesis* – The primary event in this process (Fig 1A) is the pumping of sodium without water from the ascending limb of the loop of Henle (5 11 12, 17) As a result, the molality of fluid in the descending limb rises while that in the ascending limb falls. This small difference is then multiplied as illustrated in Fig 1A.

Berliner *et al* (13) and Pinter and Shohet (14) have proposed modifications of this theory in which the vasa recta act together with a sodium pump in the loop of Henle to establish the osmolar gradient in the renal medulla. In each of these theories the energy for multiplication is provided by a solute pump

(B) *Countercurrent multiplication by the vasa recta* – The principle of countercurrent multiplication by hydrostatic pressure was one of two mechanisms applied by Hargitay and Kuhn (10) to the loop of Henle. In the present theory the same principle is applied to the vasa recta (Fig 1B) It is suggested that water ultrafilters transversely from descending to ascending limbs of the vasa recta loop

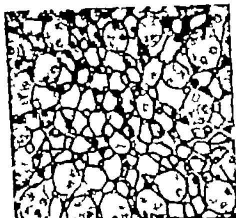


Fig 4 A. Transverse section of vasa recta bundle in the outer medulla.

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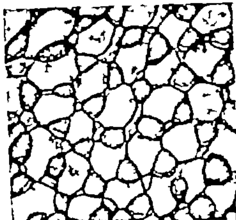


Fig 4B. Transverse section of the swim bladder rete.

In both vasa recta bundle (4A) and swim bladder rete, large venous vessels alternate with small arterial vessels in such way that artery is usually related throughout its circumference to vein and vice versa.

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the bundle. A way in which this plexus might be responsible for the dilution of fluid in the thick segment of the ascending limb of the loop of Henle is discussed in the third part of this paper (page 32)

Comparative Anatomy of the Vasa Recta

Fish, reptiles and amphibians are unable to concentrate urine (*see* 37) The main blood supply of the renal t bule in these ertebrates is a low pressure portal enous system (*see* 34) In the bird, n add tional arteriolar loop (analogous to the vasa recta) develops from the high pressure glomerular circulation (35, 43) A loop of Henle is also present in the bird (36) which, with the mammal is the only vertebrate capable of forming urine more concentrated than plasma (*see* 37) Both vascular and nephron loops, therefore are present in verte brates capable of producing concentrated urine.

The relative width of the inner zone of the medulla (or the length of the thin ascending limb of the loop of Henle) in different mammals is directly related t both the dryness of the environment and the maximum concentration of urine (38 39 40) As the concentration achieved by a multiplier is directly related to its length (10) the demonstration of an association between medullary length and urine concentration is further evidence that multiplication occurs within the medulla. It does not, however permit the conclusion that the multiplication process occurs in either the long

form compact bundles of vessels (Fig 3) These descend to the tip of the renal papilla where individual vessels unite through short capillaries with venous vasa recta which then ascend within the bundle to join the arcuate vein at the corticomedullary junction (25 27–30 61)

In transverse section, ascending and descending vessels of the bundles alternate in a way (Fig 4A) which is identical to the blood vessels of the swim bladder rete (Fig 4B) a structure known to be capable of countercurrent exchange (42) (The difference between countercurrent multiplication and exchange is considered on page 14) Scholander (33) has emphasized the importance of this alternating vascular pattern in maintaining the high transverse permeability essential for countercurrent exchange. The electron microscopic and histochemical similarity of the vasa recta and the vascular rete in the fish (31) led Longley *et al* (22) to conclude that the vasa recta might be able to contribute not only to the maintenance of, but to the creation of the hyperosmolarity of the renal papilla

Theoretically slow flow and high pressure are ideal conditions for multiplication (10) Anatomical observations suggest that such conditions may be present in the vasa recta. The efferent arterioles of juxtamedullary glomeruli (those supplying the vascular loop) are wider than those nearer the surface of the cortex (25 26 27 28 29) In some instances, afferent and efferent vessels unite directly without the interposition of a glomerular capillary plexus (24 25 28, 30) Efferent arterials then divide to

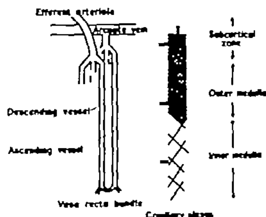


Fig 3. Schematic representation of vasa recta bundles and capillary plexus in relation to the zones of the medulla.
After Moffat and Fourman (29)

form 5–20 vasa recta the diameter of each division approaching that of the parent trunk (23 25 30) As emphasized by Trueta *et al.* (25) the vessels within the vasa recta bundle are, therefore considerably larger than post-glomerular vessels of the cortex. On entering the inner medulla, moreover the size of the vasa recta again increases (29 30) A possible consequence of these unusual anatomical features is that the velocity of flow would be reduced within the vasa recta with relatively little change in pressure.

(B) *The capillary plexus of the medulla.* – A dense capillary plexus is present in the outer medulla in addition to the vasa recta bundles (27 29 30) (Figs. 2 and 3) On entering the inner medulla the plexus becomes less dense and less clearly demarcated from the long vascular loops (29 30) (Fig 2) Its blood supply is derived from efferent glomerular arterioles and vasa recta (Fig 3) In some instances (*see* 30) one of a pair of efferent glomerular arterioles supplies the plexus, the other arteriole supplying

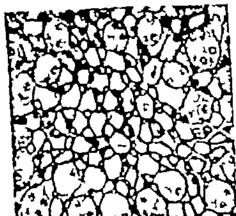


Fig. 4A. Transverse section of vasa recta bundle in the outer medulla.

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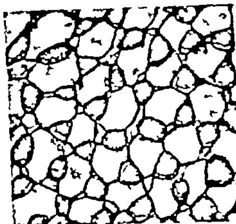


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In both vasa recta bundle (4A) and swim bladder rete, large venous vessels alternate with small arterial vessels in such a way that artery is usually related throughout its circumference to vein and vice versa.

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the bundle. A way in which this plexus might be responsible for the dilution of fluid in the thick segment of the ascending limb of the loop of Henle is discussed in the third part of this paper (page 32).

Comparative Anatomy of the Vasa Recta

Fish, reptiles and amphibians are unable to concentrate urine (*see* 37). The main blood supply of the renal tubule in these vertebrates is a low pressure portal venous system (*see* 34). In the bird, an additional arteriolar loop (analogous to the vasa recta) develops from the high pressure glomerular circulation (35, 43). A loop of Henle is also present in the bird (36), which, with the mammal is the only vertebrate capable of forming urine more concentrated than plasma (*see* 37). Both vascular and nephron loops, therefore, are present in vertebrates capable of producing concentrated urine.

The relative width of the inner zone of the medulla (or the length of the thin ascending limb of the loop of Henle) in different mammals is directly related to both the dryness of the environment and the maximum concentration of urine (38, 39, 40). As the concentration achieved by a multiplier is directly related to its length (10), the demonstration of an association between medullary length and urine concentration is further evidence that multiplication occurs within the medulla. It does not, however, permit the conclusion that the multiplication process occurs in either the long

form compact bundles of vessels (Fig 3) These descend to the tip of the renal papilla where individual vessels unite through short capillaries with venous vasa recta which then ascend within the bundle to join the arcuate vein at the corticomedullary junction (25 27–30 61)

In transverse section, ascending and descending vessels of the bundles alternate in a way (Fig 4A) which is identical to the blood vessels of the swim bladder rete (Fig 4B) a structure known to be capable of countercurrent exchange (42) (The difference between countercurrent multiplication and exchange is considered on page 14) Scholander (33) has emphasized the importance of this alternating vascular pattern in maintaining the high transverse permeability essential for countercurrent exchange. The electron microscopic and histochemical similarity of the vasa recta and the vascular rete in the fish (31) led Longley *et al* (22) to conclude that the vasa recta might be able to contribute not only to the maintenance of but to the creation of the hyperosmolarity of the renal papilla

Theoretically slow flow and high pressure are ideal conditions for multiplication (10) Anatomical observations suggest that such conditions may be present in the vasa recta. The efferent arterioles of juxtamedullary glomeruli (those supplying the vascular loop) are wider than those nearer the surface of the cortex (25 26 27 28 29) In some instances, afferent and efferent vessels unite directly without the interposition of a glomerular capillary plexus (24 25 28 30) Efferent arterials then divide to

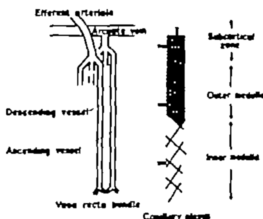


Fig 3. Schematic representation of 'asa recta bundles and capillary plexus in relation to the zones of the medulla.
After Moffat and Fourman (29)

form 5–20 vasa recta, the diameter of each division approaching that of the parent trunk (23 25, 30) As emphasized by Trueta *et al.* (25) the vessels within the vasa recta bundle are, therefore, considerably larger than post-glomerular vessels of the cortex. On entering the inner medulla, moreover the size of the vasa recta again increases (29 30) A possible consequence of these unusual anatomical features is that the velocity of flow would be reduced within the vasa recta with relatively little change in pressure.

(B) *The capillary plexus of the medulla.* – A dense capillary plexus is present in the outer medulla in addition to the vasa recta bundles (27 29 30) (Figs. 2 and 3) On entering the inner medulla the plexus becomes less dense and less clearly demarcated from the long vascular loops (29 30) (Fig 2) Its blood supply is derived from efferent glomerular arterioles and vasa recta (Fig 3) In some instances (*see* 30) one of a pair of efferent glomerular arterioles supplies the plexus, the other arteriole supplying

strated in the vasa recta. Angiographic (59 60) photocell (62, 63) and A/V transit time studies (66, 67) have shown that the circulation of the kidney is made up of several distinct parts. In some of these experiments, the large fast component (2–4 sec) of the cortex was distinguished from an intermediate component to the outer medulla and a slow component to the inner medulla. Thorburn et al. (67) have suggested that the threefold distribution was related to the anatomically distinct circulations which supply these zones.

A steep fall in hydrostatic pressure has recently been demonstrated in the descending limb of the vasa recta loop (69) (from a level possibly as high as 50 mmHg at the base, to 7 mm at the tip). As anticipated from anatomical studies, these pressures are higher than those found in cortical capillaries (70–72).

The separation of water from solute in the renal medulla. – During dehydration, sodium (Na^{22} and Na^{24}) is rapidly incorporated into the papilla (4 mins to 80% equilibration (77)) while water (THO) is excluded (60 mins to 80 equilibration (77)). As a result of this striking separation of sodium and water most of the water which would otherwise enter the inner medulla is bypassed through the outer medulla (see 16 and 51). This process could theoretically result from interstitial hyperosmolality created in the outer medulla by the pumping of sodium without water from the loop of Henle. However the amount of sodium available at the tip of the loop (less than 20 of the filtered sodium in the hamster (8 57)) seems insufficient to provoke transverse water movement to this extent.

The alternative explanation suggested by the present theory is that the water bypass results from its ultrafiltration across the top of the vascular loops, and that as such it represents the primary event of multiplication by the vasa recta (a bypass of water across both vascular and nephron loops was proposed by Morel et al. (51)). This conclusion is supported by the observation that the concentration of plasma protein within the vasa recta increases towards the tip of the papilla (73–75 and 272) a change which was attributed to the loss of water from the descending limb of the vascular loop.

The hypothetical basis of this separation of water and sodium is the greater transvascular permeability of the vasa recta to water than sodium. Such a small difference in permeability (or extravascular distribution) has been demonstrated in the circulations of the kidney (64 65) the lung (76, 78) the forearm (79) and the hindleg (64) of various mammals. While this difference is probably insufficient to produce measurable osmotic gradients in these sites, its existence in the vasa recta loop might permit a small primary effect (the separation of water from its major solute) to be multiplied to measurable proportions.

Preglomerular excretion of sodium and water. – When isotope sodium (Na^{22}) and water (DHO or THO) are injected together with a marker of glomerular filtration (inulin or creatinine) both sodium and water appear in urine before the marker

loop of Henle or the vasa recta as both these structures are of comparable length

Anatomy of the Loop of Henle

In contrast to the appearance of the vasa recta, serial section of the medulla (22, 25 34) does not suggest that the limbs of the loop of Henle are related to one another in the pattern of a counter current mechanism. Moreover the appearance of the squamous-epithelial cells of the thin segment of the loop of Henle is not that usually associated with the active transport of solute (20 41)

Summary The form of the vasa recta bundle is indistinguishable from that of known countercurrent exchangers. Microscopic and comparative studies suggest that the hydrostatic pressure may be higher in this than in the capillary circulation of the cortex. By contrast, the loop of Henle is not arranged in the manner of known countercurrent systems.

The Vasa Recta in Relation to the Solute Gradient of the Medulla

Wirz, Hargitay and Kuhn (2) first demonstrated the steep gradient of molality in the renal medulla of the dehydrated rat. This was the first experimental support for the suggestion (1) that a countercurrent mechanism was responsible for the concentration of urine. The main solute components of this gradient are sodium chloride and urea (44–55) (Fig 5) It has further been shown that during dehydration, fluid within the loops of Henle (8 57) vasa recta (3) and collecting ducts has approximately the same molality at any

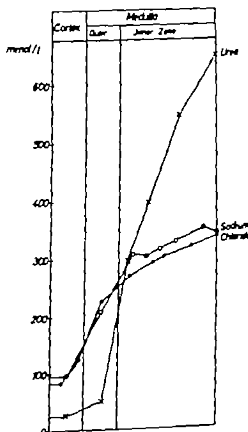


Fig. 5. Sodium, chloride and urea concentrations in kidney slices from cortex and medulla. Experiments on three dogs with similar urine osmolality (1 73, 1 66, 1 64) The three solutes account for 75 of the osmotic pressure within papillary tissue.

From Ullrich *et al.* (17) Reproduced by permission of the publishers of *Progress in Cardiovascular Disease, 3* 395–431

transverse level While these and other studies have shown that urine is concentrated by the reabsorption of water from the collecting ducts into the hyper tonic medulla the means by which the hypertonicity is established in the first place is less certain.

Pressure and velocity of flow in vasa recta. – Theoretically a slow flow and a high pressure are ideal conditions for multi plication (10) Both have been demon-

(17 89 103 107) to explain the low oxygen saturation of urine (104 105) and the presence of reverse oxygen gradient in the medulla (102, 103 106)

The opposite effect, that of a barrier to the exit of diffusible material, would result if it were added t the papilla (Fig 6B) Berliner *et al.* (13) suggested that this process might explain the trapping of urea in the papilla Similarly the high pCO_2 of alkaline urine (90 91 has been attributed to its production in the medulla by oxidative metabolism nd trapping by exchange in the vasa recta (17)

Summary The slow rate of flow through the vasa recta loop, and the large pressure difference between the limbs of the loop are ideal conditions for multiplication by pressure The exclusion of water from the i ner medulla, and the loss of water from the descending limb of the vascular loop, may represent the primary event in multiplication by the vasa recta.

The ability of the vasa recta to act as an exchange barrier to diffusible substances lso suggests that if the barrier effect were greater for water than sodium, water exchange might combine with sod m trapping t produce sodium multiplic tion.

The Effect on Urine Concentration of Acute Changes in the V sa Recta Circulation

A mportant aspect of the urine concentration mechanism is that it should b capable of rapid modification in response to changes in sol te and water intak If multiplication occurs within the vasa recta as suggested, an acute increase in concentration could theoretically (10) be produced by either an increase in pressure or a decrease in flow the opposite changes would result in a decrease in concentration.

Kramer (58) Pinter and Shohet (14) and Gunzler (32) have recently analysed the effect of change in vasa recta flow in a theoretical model based on a sodium pump in the loop of Henle. While these analyses are generally in close agreement with experimental data relating asa recta flow and urine concentration (32, 63) a relationship such as this might also be anticipated in process based on multiplication by the vasa ecta alone. Other studies (5 256) also indicate that the medullary gradient might be affected by the rate at which blood or tubular fluid passed through the renal medulla.

I the following section it is proposed to consider a way in which vasoactive substances might affect urine concentration as a result of a direct action on the vasa recta

Vasoactive Substances and Urine Concentration

In small amounts most vasoconstrictor substances produce an antidiuresis and sodium retention (antinatriuresis) The opposite effects are usually produced by vasodilators. These hanges have the same timing as other vasoactive manifestations, and can occur without measurable change in glomerula filtration rate or blood pressure. The present theory suggests that the antidiuretic effect results from the constriction of the vasa recta, and diuresis from their dilatation.

(80 81 82, 86) A similar preglomerular component of excretion has been noted with chloride (81) potassium (83 86) ammonia (84) CO_2 (85) calcium (86 100) and strontium (86) Chinard and Enns (81) and others have suggested that the most likely explanation of the preglomerular excretion of sodium was the existence of some form of vascular–nephron bypass in which sodium was delivered to the tubule ahead of glomerular filtrate [1] In the present hypothesis it is suggested that a transfer of this type occurs between the vasa recta and the loop of Henle, and that as a result, these diffusible substances enter the renal tubule ahead of material which has been filtered by the glomerulus (inulin) and passed through the proximal tubule

Distribution of other solute in the renal medulla. – The ability of chloride (44 45) bromide (87) and iodide (88) to form concentration gradients in the medulla during dehydration may be the result of their association as anions with sodium Gradients of creatinine, calcium and phosphates have also been described (47)

In contrast to these gradients a second group of substances (K Rb Kr and O_2) are distributed in the opposite manner as a reverse gradient, the minimum concentration being present at the tip of the papilla. A way in which both types of gradient may be related to the countercurrent mechanisms of the medulla is discussed in the following section.

The principles of countercurrent multiplication and exchange are similar

[1] See (86) for discussion of alternative mechanisms

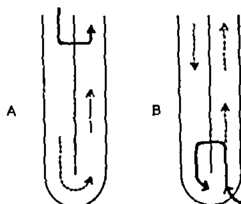


Fig. 6—Vasa recta loop as countercurrent exchange barrier

A. Substances with a greater transvascular permeability than sodium (K, Rb, Kr and O) bypass the loop to relatively greater extent than sodium and are excluded from the inner medulla

B. Opposite effect when any diffusible substance gains access to papilla (e.g. by reabsorption from collection duct)

(*see* 20 21) If multiplication occurs in the vasa recta as proposed exchange might also occur Lassen and Longley (89) have suggested that the vasa recta act as a countercurrent exchanger providing a barrier to the net transport of material along its long axis. The more diffusible the material the more effective the barrier The barrier could be effective in either direction. A barrier to the entry of diffusible material (Fig 6A) might as has been suggested by others, explain the exclusion of potassium (83 88 92, 93) rubidium (87 94) and krypton (67 89 95) from the medulla and the tendency of these substances to form reverse gradients (concentration lowest in papilla) (47 67 94 97) This process might be attributable to their highly diffusible nature (98 101) A similar mechanism has been proposed

(17, 89, 103, 107) to explain the low oxygen saturation of urine (104, 105) and the presence of a reverse oxygen gradient in the medulla (102, 103, 106).

The opposite effect, that of a barrier to the exit of diffusible material, would result if it were added to the papilla (Fig. 6B). Berliner et al. (13) suggested that this process might explain the trapping of urea in the papilla. Similarly the high pCO_2 of alkaline urine (90, 91) has been attributed to its production in the medulla by oxidative metabolism and trapping by exchange in the vasa recta (17).

Summary. The slow rate of flow through the vasa recta loop and the large pressure difference between the limbs of the loop are ideal conditions for multiplication by pressure. The exclusion of water from the inner medulla, and the loss of water from the descending limb of the vascular loop, may represent the primary event in multiplication by the vasa recta.

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Paradoxically large amounts of vasoconstrictor substances often produce a diuresis and natriuresis (the opposite effect to small amounts) It is suggested that this concentration reversal[1] is also an effect of vasoconstriction.

Hypothetical mechanism of concentration reversal – If the velocity of flow in a multiplier is reduced the concentration effect is increased (10) As was pointed out, however (10) this increase is limited to a maximum by the diffusion of water and solute in the long axis of the multiplier If flow is reduced beyond this maximum, the concentration effect might then decrease (as the speed with which the gradient could be established would be less than its speed of dissipation) until at infinitely slow flow (death) no concentrating effect would exist. It is, therefore, theoretically possible that an optimum flow rate exists from which both increase and decrease of flow produce a decrease in concentration (Fig 7) In this way progressive vasoconstriction might lead at first to an increase in concentration and subsequently (with further constriction) to a decrease in concentration.

While no direct experimental support for this suggestion is known, the tendency for the solute gradient to dissipate by longitudinal diffusion must be considerable. The desert rat, for example can concentrate urine to 5000 mOsm/Kg (40) This implies the existence of a

[1]The term 'concentration reversal' has been used in preference to 'volume reversal' or 'sodium reversal' Although V and U_{Na} V usually reverse together in the various situations to be described, a dissociation does occur in some instances (e.g 112, 143, 144 145)

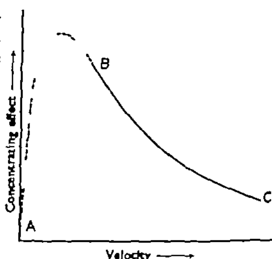


Fig 7 Hypothetical relation between velocity of flow and concentrating effect in a multiplier

Part B.C. of curve has been demonstrated experimentally (see Fig 13 of Thurau (68)) The different form of curve A.B. is hypothetical consequence of the increasing effect of longitudinal permeability at slow flows. This limiting effect was discussed by Hargitay and Kuhn (10).

gradient rising from approximately 300 mOsm/Kg in the cortex to 5000 mOsm/Kg in the papilla over a distance of 5 mm (39) (950 mOsm/Kg/mm of distance) The presence of transverse interstitial bars in the papilla of this species (193) may have the effect of partitioning the interstitial space transversely thereby reducing longitudinal diffusion and increasing concentration capacity

In the following sections, these theories are considered in relation to the effects of vasoactive substances on urine concentration

Acute effects of angiotensin and renin on urine concentration – Infusion of angiotensin at rates between 0 003 and 0·03 microgram/Kg/min in the dog produces a marked antidiuresis and antinatriuresis. With higher rates of infusion (0·06–0 13 microgram/Kg/min) diuresis and natri-

uresis result (109). This reversal of effect was not related to an increment in blood pressure. A similar phenomenon has recently been described in the rabbit (110, 111). Earlier experiments in the dog, during a water diuresis (112) showed that the sodium retaining effect of small amounts of angiotensin reversed with larger amounts to produce a natriuresis. This reversal might be the basis of the antidiuretic or sodium-retaining effects of angiotensin and renin reported in some circumstances (113–121, 130, 144) and of the diuretic or natriuretic effects reported in others (113–116, 120, 122, 131). The possibility that factors other than dose are invol ed in this concentration reversal is discussed later (page 19).

A small amounts of angiotensin stimulate ldosterone secretion (see 132) t might seem possible that the effect of angiotensi on sodium excretion was mediated by aldosterone. That this is unlik ly however is show by the per istence of the effect in adrenalectomized patients (139) and the ipsilateral changes which occur when angiotensin is infused t one renal artery (133). Furthermore, neither the speed of the response nor the hanges in other electroly tes are characteristic of those produced by aldosterone (109, 128). The existence of this acute effect of ngiotensin on sodium excretion does not, how ever, preclud a second more gradual effect mediated by aldosterone. Natri resis is not, for example maintained during prolonged angiotensin infusion (115, 122, 129). In some instances (115), sodium retention occurs d ring the later stages of such infusions. This secondary effect could well be the result of the increased aldosterone secretion (see 115).

Adrenaline and noradrenaline. — As with angiotensin the renal response to the smallest effective dose of adrenaline and noradrenaline is an antinatriuresis usually associated with an antidiuresis (134, 135, 136, 137). In man, the antinatriuresis may be associated with a small diuresis (143, 144, 145). The intrarenal nature of these actions of adrenaline and noradrenaline is indicated by the ipsilateral effect of infusion i to one renal artery (138).

The observation that small amounts of adrenaline are capable of reducing the velocity of blood flow through the inner medulla[1] with relatively little effect on that of the cortex (146) supports the present suggestion that the effect of vasoconstrictors on urine concentration is mediated by their effect on this circulation.

Larger amounts of adrenaline and noradrenaline generally produce natriuresis and diuresis (141, 142). This effect may to some extent be the result of a rise in blood pressure, as is shown by ts elimination during constant pressure perfusion (141, 142). A d uresis and natriuresis independent of pressure change can, how ever, follow large intramuscular injections (140), renal artery infusion (191) and intra enous infusion in various pathological states in man (120, 127, 145).

[1]The technical aspects of methods for measuring medullary blood flow hav been discussed by Thurau (4). The important variable from the point of iew of the present hypothesis is the velocity of blood flow, an index of which is obtained by the circulation time experiments.

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While no direct experimental support for this suggestion is known, the tendency for the solute gradient to dissipate by longitudinal diffusion must be considerable. The desert rat, for example, can concentrate urine to 5000 mOsm/Kg (40) This implies the existence of a

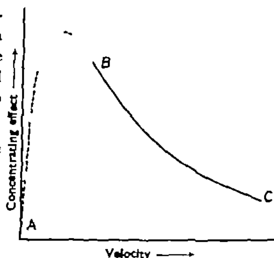


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In larger amounts most vasoconstrictors produce a diuresis which is independent of change in blood pressure. This reversal of effect may be the result of more extreme medullary vasoconstriction.

Vasodilator substances produce a diuresis independently of change in blood pressure. It is suggested that this effect results from an increase in medullary blood flow

Other factors in the concentration reversal. – The relation of the concentration reversal to the dose of vasoconstrictors has been described. This reversal can, however occur independently of dose. Both angiotensin and adrenaline, for example, produce diuresis in cirrhotic, hyper tensive or salt deprived normal subjects (120 126, 130 145) while comparable infusions in normal subjects on higher salt intake usually result in an antidiuresis (120, 130, 145) That corticosteroids may participate in some way in this reversal is further indicated by the diuresis reported in single cases of primary aldosteronism (126, 128, 190) and Cushing's syndrome (120) and by the bility of steroids to convert the antinatriuretic response to a natriuresis both in the presence (131 192) and absence (124) of the suprarenal gland.

Renal Artery Pressure and Urine Concentration

Small changes in renal artery pressure produce large changes in the volume and concentration of urine in the absence of measurable change in glomerular filtration. It is suggested that these urinary effects are, in part, the result of the alterations in medullary blood flow which are known to occur in these cir cumstances (65, 170) [1]

Effect on urine volume – Unilateral reduction of renal artery pressure in man (171 172, 173 174) and the dog (49 176, 177 178, 179 180 181) is associated with a marked reduction of urine volume on the affected side. This change has been described in the anaesthetized and conscious state, in acute and chronic experiments on the intact, denervated and isolated kidney during various rates of solute and water excre tion, and with different degrees of pressure reduction. In several instances (49 176 177 178) it has been noted with little or no change in glomerular filtration rate.

The concentration of P.A.H (U_{PAH}) Inulin (U_{In}) and creatinine (U_{Cr}) are usually increased in the urine produced by a kidney with renal artery stenosis. The relation between U_{In} and V is such, that although C_{In} falls with more marked stenosis, the decrease in V is relatively greater U_{In} (also U_{PAH} and U_{Cr}) is, therefore, usually increased on the side of a renal artery stenosis over a wide range of reduction in pressure. The consistency of this change has led to its use in the diagnosis of renal artery stenosis in man (172, 173 174)

Effect on U_{osm} and U_{Na} – During progressive reduction of renal artery pressur in the dog, the molality of urine

[1] Berliner et al (13) raise this possibility (see their footnote to page 739)

Other vasoconstrictor substances – In normal man, metaraminol is antinatriuretic, in the cirrhotic patient with ascites, natriuretic (147) In man (148) and the dog (149 150) oxytocin is slightly antidiuretic larger doses in the dog produce natriuresis (149 150) and in the rat both natriuresis and diuresis (151 152)

Antidiuretic hormone (A.D.H) – It is generally held that A.D.H produces concentrated urine by an action on the water permeability of the distal nephron (*see* 153) While A.D H has undoubted effects on the water permeability of some biological membranes (*see* 153) the direct demonstration of a permeability change[1] in the nephron has only recently become possible (*see* 156) Theories of the antidiuretic action of A.D H are, therefore, based mainly on studies of membranes outside the kidney (*see* 157)

Like adrenaline, A.D.H constricts the vasa recta, reducing the high rates of medullary blood flow present during water diuresis to the slow flow rates characteristic of dehydration (63) A D.H also converts the minor solute gradient found in the medulla during water diuresis to the steep gradient of dehydration (158) It would seem possible, therefore, that at least part of the mechanism by which A.D H produces concentrated urine is by a reduction of medullary blood flow and that this in turn leads to an increase in the multiplying effect of the vasa recta and an increase in the solute gradient.[2]

These experiments in no way exclude an action of A.D H on *tubular permeability* although as Lamdin (21) has pointed out, if such an action were its only renal effect, the increased reabsorption of water into the papilla during dehydration would reduce rather than increase the molality in this site. It may be relevant that angiotensin, a substance with relatively little effect on the water permeability of the toad's skin (56) is capable of producing an antidiuresis in patients with diabetes insipidus (108 121)

Paradoxically large amounts of A.D H (or pituitary extracts) can produce an intense diuresis (159 160 161 162, 163 164 165) In some experiments the effect was independent of change in blood pressure. It is suggested that this concentration reversal (similar to that occurring with other vasoconstrictors) is the result of extreme constriction of the vasa recta.

Vasodilator substances and urine concentration. – Infusion of acetylcholine (166 167) kallidin (168) and bradykinin (169) into the renal artery or aorta is followed by an intense diuresis and antinatriuresis. It is suggested that this effect (the opposite to that produced by vasoconstrictors) is the result of dilatation of the vasa recta.

Summary Small amounts of vasoconstrictor substances are antidiuretic The suggested mechanism of this effect a decrease in vasa recta flow has been

[1] By such criteria as have been applied to amphibia skin (154) and the toad bladder (155)

[2] Levitin *et al.* (158) suggested that part of the effect of A.D.H. on urine concentration may be t increase the trapping of sodium in the medulla by constriction of the vasa recta

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Other factors in the concentration reversal. – The relation of the concentration reversal to the dose of vasoconstrictors has been described. This reversal can, however occur independently of dose. Both angiotensin and adrenaline, for example, produce a diuresis in cirrhotic, hypertensive or salt deprived normal subjects (120 126, 130 145) while comparable infusions in normal subjects on higher salt intake usually result in an antidiuresis (120 130 145). That corticosteroids may participate in some way in this reversal is further indicated by the diuresis reported in single cases of primary aldosteronism (126, 128, 190) and Cushing syndrome (120) and by the ability of steroids to convert the antinatriuretic response to a natriuresis both in the presence (131, 192) and absence (124) of the suprarenal gland.

Renal Artery Pressure and Urine Concentration

Small changes in renal artery pressure produce large changes in the volume and concentration of urine in the absence of measurable change in glomerular filtration. It is suggested that these urinary effects are, in part, the result of the alterations in medullary blood flow which are known to occur in these circumstances (63, 170).

Effect on urine volume. – Unilateral reduction of renal artery pressure in man (1 1 172, 1 3 174) and the dog (49 176 177 178, 179 180, 181) is associated with a marked reduction of urine volume on the affected side. This change has been described in the anaesthetized and conscious state, in acute and chronic experiments on the intact, denervated and isolated kidney during various rates of solute and water excretion, and with different degrees of pressure reduction. In several instances (49 176 177 178) it has been noted with little or no change in glomerular filtration rate.

The concentration of P.A.H. (U_{PAH}) Inulin (U_{In}) and creatinine (U_{Cr}) are usually increased in the urine produced by a kidney with renal artery stenosis. The relation between U_{In} and V is such, that although C_{In} falls with more marked stenosis, the decrease in V is relatively greater. U_{In} (also U_{PAH} and U_{Cr}) is, therefore, usually increased on the side of a renal artery stenosis over a wide range of reduction in pressure. The consistency of this change has led to its use in the diagnosis of renal artery stenosis in man (172, 173 174).

Effect on U_{osm} and U_{Na}. – During progressive reduction of renal artery pressure in the dog, the molality of urine

Berliner et al. (13) raise this possibility (see their footnote to page 739)

from the affected kidney at first increases above that of the control kidney (49 *181*). With further pressure reduction the effect reverses and molality decreases on the affected side (49). Similar changes can occur in urine sodium, a small reduction in pressure leading to an increase in U_{Na}, while a larger reduction leads to a decrease (177, 178).

During progressive reduction in renal artery pressure, therefore, U_{osm}, U_{Na}, U_{PAH}, U_{In} at first rise while V falls markedly. With more marked reduction in pressure, U_{osm} and U_{Na} fall, the remaining changes persisting. It is suggested that the increase in concentration (U_{osm} and U_{Na}) is the result of the reduced medullary blood flow demonstrated in these circumstances (63, 170) and that the subsequent decrease in concentration is the result of a combination of reduced pressure and reduced glomerular filtration.[1]

Reduction in renal artery pressure during diuresis – During osmotic and water diuresis the difference in the osmolality of urine produced by a stenosed kidney is increased relative to that of the unstenosed control (49, 182). This difference may be the result of increase in medullary blood flow during osmotic and water diuresis (63) in that a greater reduction in pressure is necessary in a dilated vascular bed to produce the critically slow flows necessary for maximum urine concentration.

[1]Although pressure and flow may be analysed independently in a theoretical model, it is unlikely that they would vary independently in vivo. The effect of a reduction in renal artery pressure might, as has been discussed, be a composite one of a primary reduction in pressure with a secondary reduction in flow. In the same way, the action of the vasoconstrictor substances might also be a composite effect of reduced flow (increased concentrating effect) and reduced pressure (decreased concentrating effect). The concentration reversal discussed earlier might be attributable to variations in the extent of these effects, rather than to limitation by longitudinal permeability.

Increased Renal Artery Pressure

An increase in renal artery pressure is followed by a marked increase in urine volume and solute excretion (a pressure diuresis). This effect occurs in perfusion experiments (175, 183, 184, 185, 186), follows the grafting of a normal kidney into a hypertensive recipient (189), and may in part be responsible for both the diuresis produced by adrenaline and noradrenaline (141, 142) and the diuresis which follows the correction of a renal artery stenosis (187, 188). It is suggested that a pressure diuresis is attributable to a reduction in the concentrating effect of the vasa recta, consequent upon an increased velocity of medullary blood flow. Such an increase in flow has been demonstrated experimentally (63, 170). Alternatively, it has been suggested (15, 63) that an increased medullary blood flow might lead to a diuresis by washing out the concentration gradient established by a sodium pump.

Summary. Urine volume decreases with a fall of renal artery pressure and increases with elevated pressure. It is suggested that both these effects are the result of changes in medullary blood flow consequent upon the change in pressure.

Urine concentration (U_{osm} and U_{Na}) is increased by a small reduction in renal artery pressure. This may also be a

consequence of the reduced medullary blood flow Greater reductions in pressure produce a decrease in concentration. This could result from either the pressure reduction itself, or from a reduction in glomerular filtration.

Autoregulation of Total Renal Blood Flow and Glomerular Filtration

Small changes in renal artery pressure within the range of 80–160 mmHg produce marked changes in the volume and concentration of urine (178, 183 184 186) in the relative absence of change in renal blood flow or glomerular filtration (176–178, 183, 186, 194 195, 197 200)

While this autoregulation of renal blood flow is apparent in the cortical circulation, it does not occur in the renal medulla, where blood flow changes in response to alteration in renal artery pressure (63 170) It is suggested that this dissociation of the effect of pressure on the cortical and medullary circulations is the means by which the mammal varies the concentration of urine in response to pressure change without affecting glomerular filtration. This attribute could be important if, as has been suggested (201) small changes in renal artery pressure were determinant in the homeostatic control of sodium and water

Plasma skimming and regional autoregulation. In 1956, Pappenheimer and Kinter (194 202, 203) suggested that the autoregulation of total renal blood flow might be attributable to plasma skimming in the interlobular artery As a result of this process, the haematocrit (and viscosity) of blood entering the cortex would be high, while that entering the medulla would be low The existence of plasma skimming and its variation with pressure have been confirmed in models (see 204) An anatomical basis for skimming exists in the angle at which afferent glomerular arterioles branch from the interlobular trunk (29)

This theory has not been generally accepted, mainly as a result of the failure to confirm the haematocrit gradient. These haematocrit measurements were, however based on the ratio of labelled red cells to labelled protein in kidney slices. As considerable evidence suggests that protein rapidly gains access to renal E.C.F., and that red cells do not (206 207 216) such haematocrit measurements cannot be accepted as a valid objection to plasma skimming. Furthermore, recent direct measurement of haematocrit by vessel puncture indicates that values for the cortex (208) are higher than those of the medulla (209)

If plasma skimming does occur therefore, it might account for regional autoregulation (the autoregulation of cortical blood flow in the absence of autoregulation of the medullary circulation (63)) by a process in which regional haematocrit and viscosity change as a result of alterations in renal artery pressure (see 204) It is not known, how ever to what extent this process could account for the autoregulation of total renal blood flow as originally described (202)

Plasma skimming may have functions other than autoregulation. It might, for example, provide a bypass by which red

blood cells avoided the extremes of molality pH oxygen and CO_2 saturation encountered in the papilla

Summary Dissociation of the effect of pressure changes on G F R. and urine volume may result from regional autoregulation Regional autoregulation may itself be the result of plasma skimming

Urine Concentration and Sickle Cell Disease

Vascular occlusion is a major feature of sickle cell disease. Necrosis of the renal papilla has been reported (210 211) Independently of such lesions, however many patients with sickle cell disease are unable to concentrate urine normally (*see* 212) Perillie and Epstein (213) have recently suggested that this defect results from changes in vasa recta flow produced by the *sickling of red cells in the* anoxic and hypertonic environment of the renal medulla. This suggestion is in close agreement with the present theory

PART II

THE LOOP OF HENLE AND THE EXCRETION OF UREA

A hypothetical role for the vasa recta in the concentration of urine has been discussed. This theory implied that the loop of Henle was not involved in the concentration process. The present section is concerned with an alternative role for the loop the excretion of urea into concentrated urine.

Tubular reabsorption of sodium, water and urea during dehydration. – Considerable evidence suggests that sodium is actively' reabsorbed against an electrochemical gradient from proximal and distal convoluted tubules and that water follows as a secondary osmotic effect. As a result, urea becomes concentrated, and leaves the tubule by diffusion down its own chemical gradient (*see* 18 and 19)

Sodium, water and urea are also reabsorbed from the collecting duct (7 8, 9 162) In this site, however the reabsorption of water occurs in two ways one component results from sodium reabsorption (as in the convoluted tubules) the other from diffusion into the hypertonic renal medulla. The energy required to reabsorb this second osmotic component' (analogous to the $T^{c}_{H_2O}$ of Wesson and Anslow (215)) is spent by the mechanism which creates the osmotic gradient. As esult of this partition, water eabsorption can be controlled independently of sodium by effects acting on the 'osmotic component

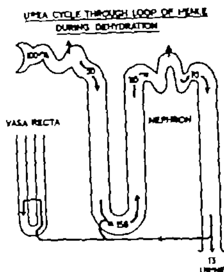


Fig. 8. Schematic representation of the movement of urea in the renal tubule. Numbers refer to the approximate percentage of filtered urea present at individual points of the nephron. The value for the loop of Henle is derived from the hamster other values are from the rat (Lassiter *et al.* (8)).

The loop of Henle and urea exchange – During dehydration a relatively large amount of urea is reabsorbed from the collecting duct (8 9 196) The concentration of urea at both the tip of the loop of Henle (8 57) and in the early distal tubule (8, 9) is greater than can be accounted for by direct entry from the proximal tubule (8, 9) These observations indicate that urea moves in a cycle (8 9 17 227) (Fig 8) from collecting duct to loop of Henle, returning a second time to the collecting duct from which point the cycle can be repeated.

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Urea Clearance (C_{urea}) during transitional states of urine flow – C_{urea} rises during periods of increasing urine flow ("the exaltation of urea clearance") and falls when urine flow is decreasing ("abatement of urea clearance") (*see* 219) A possible explanation of these changes based on the suggestions of others (47 50, 219 220, 237) is illustrated in Fig 10.

During the transition from low to high urine flow (time A–B) urine volume (V) rises rapidly The urinary concentration of urea (U_{urea}) however falls more slowly as a result of its equilibration with the large pool of urea within the medulla. The difference in the rate of change of V and U_{urea} produces temporary exaltation of urea clearance $\left(\frac{U_{urea}V}{P}\right)$ During the reverse transition – from high to low flow – (time C–D) the reverse sequence occurs (abatement) The rate of rise of U_{urea} is delayed (relative to rate of fall in V) by equilibration with the low urea content of the medullary pool.

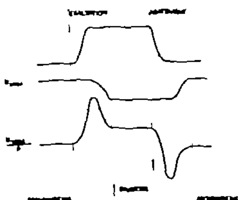


Fig. 10. Exaltation and abatement of urea clearance during transitional states of urine flow

See text Exaltation of urea clearance $\frac{U_{urea}V}{P}$ produced by relatively early increase in V as compared with fall of U_{urea}. Abatement of urea clearance produced by relatively early fall in V

Effect of the U on Exchange on Maximum Urine Concentration

As Gamble *et al.* (224) pointed out, urea is used in some way during its excretion to conserve water The maximum osmolality of urine is, for example reduced by protein deprivation (48, 221 223) This defect is both corrected (48, 221) and prevented (223) by urea. Similarly the eabsorption of solute free water ($T^c_{H_2O}$) and the concentration of urine are greatest when urea is the principal urinary solute (224 225, 226, 228) This property of urea has been attributed to its reabsorption from the collecting duct into a medullary gradient (13 230 231, 232) a conclusion which is borne out by the observation that methyl urea and acetamide form gradients (234) and augment urine concentration (233) while thiourea (a closely related substance) is less able either to enter a gradient (231 234) or augment urine concentration (233) This effect of urea may therefore, result from its reabsorption from the collecting duct, which in turn liberates further water for reabsorption (as with the reabsorption of any solute) The efficiency of this process would be considerably increased by the return of urea to the collecting duct without water (Fig 8) from which point the cycle could be repeated

Urea Exchange Cycle and the Relation of Sodium and Urea

During dehydration, the molality of the renal papilla and urine are approxi-

In effect, reabsorbed urea is re-excreted into the loop of Henle, thereby augmenting urea clearance. The efficiency of this cycle may be increased by the countercurrent exchange of urea in the vasa recta. As suggested by Berliner *et al.* (13) a trapping effect could account for the high concentration of urea found in the medulla during dehydration (47 48 50 52, 55) (Fig 5) The same process could also account for its high concentration in vasa recta plasma (74) In this way urea exchange augments the concentration of urea at the mid-point of the nephron and thereby affects 3 related processes – urea clearance, sodium reabsorption and urine concentration These relationships will be considered in the following sections.

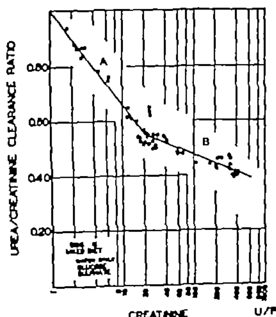


Fig 9 The relation between the proportion of urea (urea/creatinine clearance ratio) and the proportion of water (creatinine U/P ratio) reabsorbed from the renal tubule

From Smith, H. W 'The Kidney' reproduced by permission of Oxford University Press.

Augmentation of Urea Clearance by the Urea Exchange Mechanism

During dehydration, the proportion of water and urea reabsorbed from the collecting duct is increased (8 9) Oliguria therefore tends to produce uraemia By means of urea exchange, however the large fraction of urea reabsorbed from the collecting duct is subsequently re-excreted into the loop of Henle, thereby augmenting urea clearance and avoiding uraemia.

The extent of the augmentation is greatest during dehydration and least during diuresis. The reabsorption of urea from the collecting duct is, for example, markedly reduced by saline (217) and mannitol (9) diuresis. This observation may in part explain the reduction of the urea gradient (49 53) and the reduced concentration of urea in the distal convoluted tubule (9 217) in these circumstances. In this way therefore, the augmentation of urea clearance by exchange is greatest during dehydration when urea clearance would otherwise tend to be at its lowest

As noted by Shannon (218 and 222) and Smith (37) a non-linear relationship exists between the amounts of water and urea reabsorbed from the tubule (Fig 9) Both authors considered that the different slope during oliguria (slope B) was attributable to some process linked with the reabsorption of water in the distal nephron. It is suggested that this water dependent process is urea exchange, and that the departure from linearity is the result of the progressively greater exchange during oliguria.

extent inversely related (*see* 53)

The use of urea during sodium reabsorption may be important in states of sodium deprivation in that the provision of a sufficiently large amount of urea in collecting duct urine enables the sodium pump to reduce urine sodium without affecting urine molality. The provision of this urea may in turn be an attribute of the loop of Henle.

Relation Between C_{urea}, U_{urea} and U_{Na}. The Utilization of Urea During Its Excretion

In previous sections three aspects of urea excretion have been described: the maintenance of urea clearance, an effect of urea on maximum urine concentration, and the ability of urea to act as an osmotic alternative to sodium during sodium retention. A common factor in these otherwise dissimilar processes is the presence of a high concentration of urea in collecting duct urine. It is suggested that the loop of Henle maintains this high concentration, and that for this reason, relationships exist between the loop of Henle on the one hand and the excretion of urea, the concentration of urine and the reabsorption of sodium on the other.

The rare condition cystic disease of the renal medulla, often presents clinically with a combination of these three features as uraemia, sodium depletion and hyposthenuria (*see* 241). Strauss (241) suggested that the hyposthenuria in such cases might result from disorganization of the countercurrent mechanism by the cystic spaces and fibrosis surrounding the collecting ducts. This view is in close agreement with the present theory which further suggests that the sodium loss and uraemia might also result from these pathological changes.

Urea is used by the organism in such varied processes as ammonia disposal, osmoregulation and protein synthesis (*see* 37, 240, 253). In the present context, the augmentation of urine concentration and sodium reabsorption may represent further exploitation during its excretion (*see* 224).

Protein deprivation and the loop of Henle. – Urea clearance falls during protein deprivation (*see* 219) to levels which, in the herbivorous sheep (237) and camel (238), are less than 10% of the G.F.R. As ruminants such as these can synthesize protein from the urea present in blood (239), a mechanism for the retention of urea during protein deprivation would be advantageous. During protein deprivation in the sheep, the concentration of urea in both papilla and urine falls markedly, the highest concentration being present in the outer medulla (Fig. 13) (50).

A possible explanation of this change is illustrated in Fig. 14. During protein deprivation (Fig. 14A) the concentration of urea in plasma and tubular fluid falls. Countercurrent exchange of the small amount of urea entering the collecting duct is sufficient to reduce the concentration in urine. In this way the greatest concentration of urea is present in the outer zone of the medulla where its transverse movements from collecting duct to the thin segment of the loop of Henle is first possible.

During normal protein intake (Fig. 14B) the same mechanism becomes

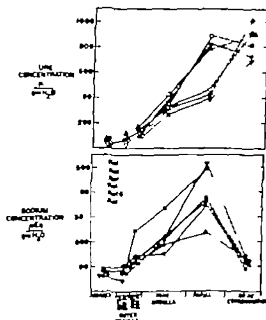


Fig 11 Distribution of sodium and urea in renal tissu and in urine of the dehydrated rat on normal protein intake Urea concentration is slightly higher in the urine than the papilla. Sodium distribution is th reverse.

From Bray and Preston (52) Reproduced by permission of *The Journal of Clinical Investigation.*

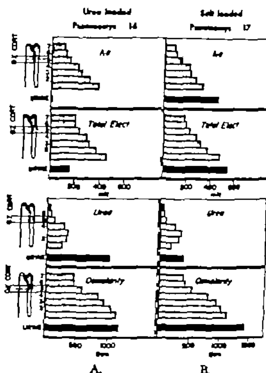


Fig 12. Concentration of urea and electrolytes i urine and in slices of kidney

A. During a urea load.

B. During a sodium chloride load.

From Schmidt-Nielsen *et al.* (53) Reproduced by permission of the *American Journal of Physiology*

mately the same. The relative amount of sodium and urea in these two sites, how ever seems inversely related (Fig 11) This reverse distribution of individual solutes between two compartments whose total solute content is the same, resembles a Donnan equilibrium of ions and may like such a state, be attributable to an active process or permeability factor affecting one component. In this instance a sodium pump in the collecting duct might reduce the concentration of sodium in urine and raise its concentration in the papilla The resultant distribution of urea would be the reverse of sodium, while the total osmolality would remain the same in both compartments. Such a process would depend upon the presence of a sufficiently large amount of urea in the collecting duct, to act as an osmotic alternative to sodium. The ability of a sodium pump to lower sodium concentration in the proximal convoluted tubule has been demonstrated with mannitol as the alternative solute (235)

Further evidence suggests an active role of sodium in this process – the inverse distribution of sodium and urea is not for example, present during sodium loading (Fig 12B) (53) a state in which the active reabsorption of sodium from the distal nephron is reduced (236) A similar mechanism may account for the observation that the concentration of sodium and urea in urine are to some

Nitrogen Excretion in Vertebrates
The excretion of nitrogen is closely related t the availability of water (240) Aquatic reptiles (242) and fresh water fish (240, 250) have a high water turnover and excrete most of their nitrogen as ammonia. In vertebrates from a drier environment, ammonia is converted int less toxic forms (uric acid, urea and trimethylamine oxid (240, 243)) and excreted as such (37 244) Uric acid – the most efficient of these substances with regard to water conservation – is excreted in semisolid form by birds and terrestrial reptiles (245 246)

Amongst the ureotelic vertebrates, the aquatic amphibian, necturus, maintains a sufficiently high urine flow to excrete urea through simple nephron. In most other ureotelic groups, however modification of (or alternative to) the simple nephron has enabled the vertebrate to excrete urea into a smaller volume of w ter More terrestrial amphibians such as the frog, actively secrete urea into tubular urine (248 249) In the teleost urea is excreted through the gills (250) while in the marine elasmobranch, without such a mechanism, urea accumulates in blood to level 2 g 100 ml) which makes an important contribution to osmoregulation (240 252

The bird nd the mammal are the only ertebrates capable of forming concentrated urine (see 37) they have therefore the smallest volume of urine av ilable for the excretion of urea. It is suggested that the development of loop of Henle by these nimals (36, 39) is the means by which urea is excreted into concentrated urine Schmidt Nielsen

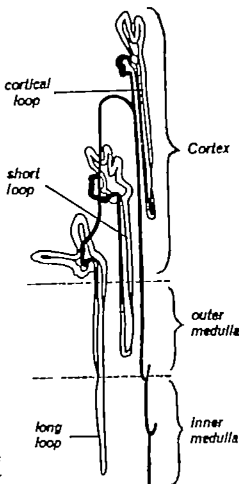


Fig. 15. Cortical, short and long loops of Henle. Adapted from Sperber (39) by kind permission.

(253) recent speculation that the countercurrent system is specifically designed for the urea excretors with a need for water conserv tion may have similar implications.

The present hypothesis is supported by the relation which exists in mammals between dietary nitrogen and the length of the loop of Henle. Of the three types (Fig 15) the long loop extending into

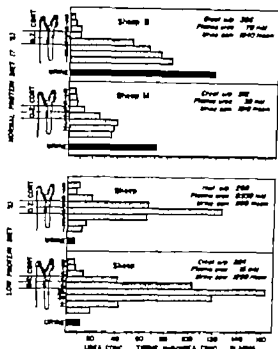


Fig 13 Distribution of urea in the kidney of sheep on low and on normal protein intake. On the abscissa is given the urea concentration in the tissue divided by the plasma urea concentration. All 4 sheep showed a constant or falling urine flow at the time just before the kidneys were removed.

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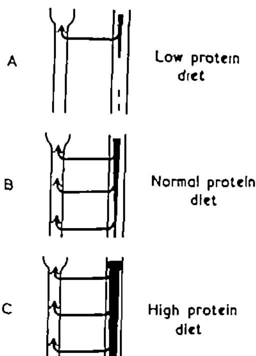


Fig 14 Countercurrent exchange of urea from collecting duct to loop of Henle (ascending or descending thin segment)

A. Complete exchange reduces concentration of urea in collecting duct and urine

B. Saturation of exchange mechanism.

C. Oversaturation (this assumes rate limited urea transfer)

relatively overloaded and transverse movement of urea makes less difference to the concentration of urea in collecting duct fluid Under these circumstances, both the transverse movement and the urea gradient extend towards the tip of the papilla.

During periods of increased protein or urea intake (Fig 14C) the exchange mechanism is further overloaded and the concentration of urea in urine rises above that in the papilla (53) (Fig 12A) Such saturation of the exchange mechanism may explain the relative failure of urea to augment urine concentration in man during a urea load (*see* discussion 232)

In this way *the loop of Henle may* act as a servomechanism, retaining urea during protein depletion, and excreting it during a protein load the type of response being determined by the amount of urea entering the system.

Exaltation of C_{urea} during protein depletion The exaltation of urea clearance is considerably greater in the sheep during protein depletion (237) That this difference might be the result of washing out the pool of urea in the outer zone (Fig 13) (50) is suggested by the rise in U_{urea} as well as C_{urea} during such exaltation.

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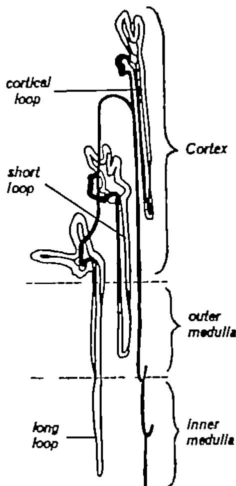


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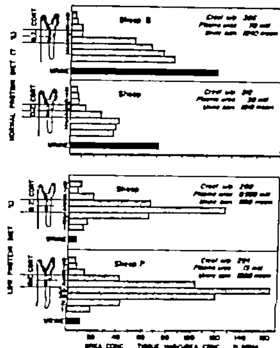


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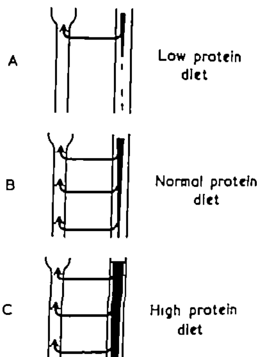


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in the medulla of two dogs given parathormone (as compared with untreated controls) (259) suggests that calcium might be involved in the countercurrent processes of the renal medulla in a manner similar to urea.[1] Under these circumstances, excessive calcium reabsorption induced by parathormone might lead to the re-cycling of calcium in high concentration through the loop of Henle and the distal nephron. Such high concentration might then lead to the calcification which occurs in these sites after parathormone (260). By analogy with the excretion of urea these changes could occur without marked change in P_{Ca}, C_{Ca} or $U_{Ca}V$.

Pathological countercurrent trapping might also occur with phenacetin and sulphonamides, substances which are known to produce lesions of the renal medulla and papilla (261). A similar process might also occur with uric acid in gout.

[1] It is not, however, implied that the mechanism of their reabsorption from the collecting duct is similar.

the inner medulla would seem most capable of participating in urea exchange. Sperber's (39) observation that the long loop occurs most commonly in the carnivores from a dry environment may therefore, be related to the need of these mammals to excrete the largest amount of urea into the smallest volume of urine. The converse observation (39) that the long loop was least common in the herbivores from the wet environment may be explicable on the same basis

Sperber (39) also noted a relationship between the form of the collecting duct and diet. A direct junction type of duct (Fig 16) was commoner in the carnivore As the concentration of urea in the renal cortex is low (47) the short course of the direct junction duct in the carnivore may serve to minimize the loss of urea by diffusion into the cortex. Conversely the arcade type of duct (Fig 16) more commonly found in the herbivore (39) may promote the diffusion of urea into the cortex, and thereby serve as a means of urea retention.

Summary Urea has been shown to move in a cycle through the renal medulla. In effect this cycle increases the concentration of urea in collecting duct fluid and thereby augments urea clearance the concentration of urine and the reabsorption of sodium. Comparative studies suggest that the long loop of Henle, an integral part of this cycle, has developed in species excreting a large amount of urea into a small volume of urine It is also possible that the same type of mechanism may retain urea during protein deprivation

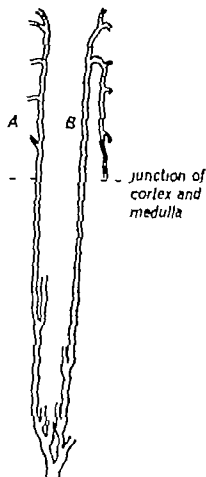


Fig. 16. Two types of mammalian collecting duct.

A. Direct junction type of collecting duct in which the juxtamedullary nephron has a very short course in the cortex.

B. Arcade type with long course in cortex.

From Sperber (39) by kind permission.

Calcium

During dehydration a gradient of calcium is present in the renal medulla (47) Micropuncture studies have shown that calcium is reabsorbed from the collecting duct, and that its concentration in fluid from the tip of the loop of Henle is greater than that in plasma or adjacent collecting duct urine (258) These observations together with the demonstration of a marked gradient of calcium

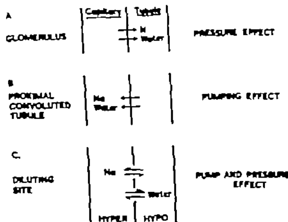


Fig. 17 Three types of relation between capillary and tubule.

A. Isotonic filtration of fluid from capillary to tubule.

B. Isotonic movement of water from tubule to capillary.

C. Combined effects produce hypotonic fluid within renal tubule.

nates (pumping or filtration) the net effect would be that the concentration of sodium (and molality) in tubular fluid would be reduced.[1] It is suggested that a process of this type might be responsible for the dilution of fluid in the thick segment of the ascending limb of the loop of Henle as a result of a difference in pressure between this part of the renal tubule and the dense capillary plexus of the outer medulla.

This suggestion differs from the conventional sodium pump hypothesis only inasmuch as its 'relative water impermeability' is an attribute of hydrostatic pressure rather than of some intrinsic quality of the ascending limb epithelium. The importance of this distinction lies in the ability of a change in capillary pressure to affect urine dilution. The observation (205) that A.D.H. can reduce the pressure within the distal convoluted tubule may be relevant in this context.

The theoretical principle of sodium pumping between compartments at different hydrostatic pressures has been applied recently to the movement of water against its gradient in the gut (278 279 280). Although the model proposed by Curran and Macintosh

[1]The concentration of sodium in the adjacent vasa recta would be increased as a result of this pressure dilution mechanism. The increase would be greater in the descending limb of the vascular loop as its pressure would be higher than that of the ascending limb. A small difference in sodium concentration could, therefore, be created between the adjacent limbs of the vasa recta loop. This might then constitute the 'primary effect' and initiate countercurrent multiplication by the vasa recta in a manner similar to that proposed by Pinter & Shohet (14).

PART III

URINE DILUTION

Fresh water vertebrates usually maintain a higher osmotic pressure than their environment by forming dilute urine (240) Comparative (244) and micropuncture studies (5 11 264 265 266) indicate that the process of dilution has developed in close association with the distal segment of the renal tubule. Three hypothetical mechanisms by which urine could be diluted in this segment will be discussed.

The Sodium Pump Hypothesis

The sodium pump hypothesis (Fig 1) suggests that the hypotonicity of early distal tubular fluid and thence the dilution of urine, are attributable to the pumping of sodium through the water impermeable ascending limb of the loop of Henle. The same process is, therefore, capable of producing both concentrated and dilute urine. Experimental evidence relating to this theory is largely indirect. While a sodium pump may be present in the thick segment of the ascending limb (*see* 18 267) its existence in the thin segment has been questioned (269 270)

An Alternative Hypothesis

The effect of sodium pumping across a gradient of hydrostatic pressure – In the amphibian, tubular fluid is diluted in the early part of the distal segment (264) The arteriolar nature of the blood supply to this segment (266 273, 274) contrasts with the low pressure portal system which supplies the remainder of the tubule (273) The diluting site in the mammal, the thick segment of the ascending limb of the loop of Henle, is similarly surrounded by an arteriolar-capillary plexus, whose pressure may well be higher than that surrounding proximal and distal convolutions (*see* page 10) A gradient of hydrostatic pressure may therefore, exist between the peritubular capillary and tubular lumen in the diluting segment of the renal tubule. A way in which such a gradient may be combined with a sodium pump to produce dilute tubular fluid is illustrated by three examples of the relation between the nephron and its blood supply

In the first, glomerular filtration (Fig 17A) a high pressure capillary is related to a low pressure tubule isosmotic filtration of water and solute result.

In the second the proximal convoluted tubule (Fig 17B) a low pressure capillary is related to a comparably low pressure tubule. In this instance, isosmotic sodium and water movement occur in the opposite direction as a result of sodium pumping

The third example the diluting segment (Fig 17C) is a hypothetical combination of the first and second examples in which sodium is pumped from a low pressure tubule into a high pressure capillary Whichever process predomi-

peritubular capillary pressure in this segment is higher than elsewhere. A mechanism of urine dilution based on this observation is discussed.

The Renin-angiotensin System

Considerable evidence (*see* 214–229) now suggests that renin is located within the juxtaglomerular apparatus (Fig. 18) and that the renin-angiotensin system is involved in some way in the regulation of sodium balance (23, 132, 283). The mechanism of this regulation may well be in the form of a feedback system (23, 284) in which renin (and thence angiotensin) stimulates aldosterone secretion and thereby produces sodium retention. In some way sodium retention then suppresses renin release.

Of these steps, the factors controlling the secretion of renin are least understood. Several hypotheses have been proposed. In these, the stimulus to renin release has variously been considered as 'renal ischaemia' (285), a change in pulse pressure (286) or mean pressure (287) in the renal artery, and a change in some factor related to the stretch, distensibility or compliance of the renal vasculature, or even of the kidney itself (276, 277, 288, 289). Others have suggested that the receptor is not in the afferent arteriole, but the closely related macula densa (Fig. 18) and that change in the composition of fluid (e.g. molality or sodium concentration) in the early distal tubule is the stimulus to renin release (68, 262, 263, 268, 271–275).

The concentration of renin in plasma is related to two main variables – sodium balance (262, 283) and some factor related to alterations in the renal circulation (257–287). Theories of the mechanism controlling renin release should, therefore, account for both variables.

We have recently suggested (262) that the stimulus to renin release might be a change in the molality of fluid in the early distal tubule, and that the decrease in molality which occurs in this site during a sodium load (5) provides the signal to the macula densa which inhibits renin release.

The present hypothesis of urine dilution provides a theoretical mechanism by which change in renal artery pressure might affect renin release in the same way. An increase in renal perfusion pressure might, for example, lead to an increase in pressure in the capillary plexus of the medulla. This would then reduce the molality of ascending limb fluid, and thereby inhibit renin release by an identical mechanism to that of sodium loading. The converse state, a reduction in renal artery pressure, might lead to an increase in molality which could in turn stimulate the release of renin. Under these circumstances, small changes in renal artery pressure (within the range in which glomerular filtration autoregulates) might lead to changes in renin release (*see* 287) and sodium balance.

The amount of renin associated with glomeruli increases from the corticomedullary boundary to the capsule of the kidney (247–251, 254). This distribution may be related to a gradation of the stimulus to renin production in such a way that the molality of fluid in contact with the maculae densae from the deep

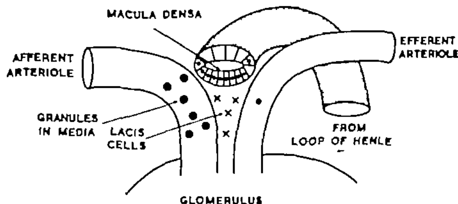


Fig 18. Schematic diagram of juxtaglomerular apparatus. Granular cells in media of afferent arteriole, lacis cells and macula densa.

(280) differs from the present one in several respects (direction of water movement, number of compartments) it is nevertheless possible that the 3 compartment system tested by them might be applicable to the present situation In such an application pressure applied to their compartment C (the peritubular capillary) could affect the rate at which water left their compartment A (the tubule) in much the same way as pressure applied to the serosal surface of the gut reduces the rate at which water is reabsorbed from the lumen (282) Ullrich and Rumrich (281) have recently considered an application of this model to water movement in the proximal convoluted tubule.

Experimental support for the present theory of dilution is indirect. The arteriolar nature of the blood supply to the diluting segment in the outer zone has been described earlier This zone is of greater length relative to the inner zone, in mammals living in a wet environment (e.g beaver and aplodontia (39)) These two species usually form dilute urine and of all mammals studied, seem least capable of forming concentrated urine (96 99)

Thurau and Deetjen (32) have recently shown that the molality of urine could be reduced to levels less than plasma by raising renal perfusion pressure. As an increase in pressure is associated with an increase in medullary (but not cortical) blood flow (63) it is possible that the pressure in the outer medullary plexus rises in these circumstances, and that this in turn is responsible for a greater dilution of fluid in the distal nephron.

A further hypothetical mechanism of dilution based on the water bypass –If more water than sodium moves transversely between the limbs of the vascular loop (Fig 1) the intervascular space would become hypotonic. If the ascending limb of the loop of Henle lay within this space, and were in water equilibrium with it tubular fluid would be diluted as a result. This possibility has not been considered further

Summary Animals living in fresh water usually osmoregulate by producing dilute urine in the early part of the distal segment of the renal tubule. Considerable evidence suggests that the

CONCLUSIONS

An association between the loop of Henle and the concentration of urine has been known for some time (38, 244). When Kuhn and Ryffel (1) introduced the concept of countercurrent multiplication by a loop in 1942, the principle was not applicable to the vasa recta, as the looped nature of these vessels had not been fully appreciated at that time. Subsequently however vascular loops were demonstrated and countercurrent exchange was shown to occur in vasa recta bundles. The present theory (like others (22, 75)) suggests that countercurrent multiplication also occurs in this site and further suggests that the multiplication is unrelated to the loop of Henle. Considerable evidence indicates that the anatomical, pressure, flow and permeability characteristics essential to such a process are present in the medullary circulation. The loss of water from the descending limb of the vascular loop, its bypass across the outer medulla and the countercurrent multiplication of sodium towards the tip of the papilla are interpreted as the primary events in multiplication by the vasa recta. While some of these findings are open to alternative explanations the evidence in favour of such alternatives is scanty. Other experiments suggest that the urinary effects of vasoactive substances and of alteration in renal artery pressure may result from changes in the vasa recta circulation (rather than direct tubular or glomerular effects).

The existence of a urea exchange cycle has been clearly demonstrated by experiment. Further evidence indicates that changes in this cycle are related to changes in the excretion of urea, the concentration of urine, and the reabsorption of sodium. The present theory suggests that the cycle and its related effects represent the main function of the loop of Henle. This interpretation does not, however exclude a role for the loop in the concentration of urine, but suggests that the role is subordinate in nature and is unrelated to the pumping of sodium from the ascending limb of the loop.

A hypothetical urine dilution mechanism based on the pumping of sodium across a gradient of hydrostatic pressure has been discussed. While no direct experimental evidence supporting this suggestion is known, considerable circumstantial evidence of an anatomical or comparative nature indicates that the necessarily high capillary pressure may be present in the outer medulla.

In conclusion, it is suggested that hydrostatic pressure has been used by the vertebrate kidney as a source of energy in three ways – that the fish developed (and perhaps subsequently lost) the ability to produce a simple glomerular filtrate of plasma that the

(juxtamedullary) glomeruli was lower than that of the more superficial glomeruli as a result of their proximity to the hypothetical dilution site in the outer medullary plexus.

Following renal artery constriction in the rabbit, the amount of renin associated with single glomeruli increases in the clipped kidney (255) The pattern of this increase is such that the gradient of renin (*see above*) is reduced renin values for deep glomeruli in some instances becoming comparable with those of superficial glomeruli. This change in distribution might be related in the present hypothesis to a relatively greater increase in the molality of fluid in contact with deep glomeruli. In this way the stimulus to renin release (increase in molality) would reach a limiting maximum when fluid in the early distal tubule became isotonic with plasma. Experimental support for these suggestions, however would require a study of the relation between renal artery pressure and the molality of fluid in the early distal tubule.

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post-glomerular circulation was modified in amphibians to produce dilute urine and that further modification in the bird and mammal led to the ability to produce both concentrated and dilute urine. It is suggested that the loop of Henle has developed in association with the vasa recta as a means of avoiding the uremia which could otherwise be produced by this mechanism.

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 433

MITRAL VALVULOTOMY

A CLINICAL AND HEMODYNAMIC PRE AND POSTOPERATIVE STUDY

by

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INTRODUCTION

Since the introduction of mitral valvulotomy as an efficient method of treatment for mitral stenosis (Harken et al., 1948 Bailey 1949 and Baker et al., 1950) large number of studies have been made of its effects. Several of these studies consisted of clinical follow-ups. Systematic clinical studies on 1000 commissurotomized patients two to nine years after the operation were made by Ellis et al. (1959) and on 300 cases for periods up to 79 months by Stewart & Glenn (1959) In these studies the anamnestic data, the functional groups to which the patient belonged before and after operation, and the valvular anatomy found at operation were correlated, and factors which appeared to be determinative for the effect of the operation were analysed.

In some postoperativ studies the effect of mitral commissurotomy has been investigated by phonocardiography or electrocardiography Systematic phonocardiographic studies both before and after commissurotomy have been comparatively rare (Contberiati & Collicelli, 1954, Gallavardin & Delahaye, 1957 Sternberg et al 1957 McKusick, 1958)

Pre postoperative changes of the lectrocardiogram of mitral stenosis patients were studied by Soulié et al (1952) Gibert Queraltó et al. (1955) and Demerdash & Goodwin (1963) Effect of commissurotomy demonstrated in these studies were changes the electrical axis of the P wa e and of the QRS complex, and in the latter study some return of the postoperatis changes of the lectrocardiogram in restenosis was observed. Rodriguez—Torres et al. (1959) studied especially the effect of valvulotomy on the electrocardiographic signs of right ventricle hypertrophy

Attempts to judge the effect of commissurotomy in mitral stenosis patients by means of standardized exercise tests have been reported in only a few instances. Bruce et al. (1956) considered they could well judge the indication for operation and also the prog nosis after commissurotomy from a physical fitness index based on an exercise test on a treadmill ergometer Logan et al. (1957) carried out serial tests of exercise tolerance before and after commissurotomy in 60 patients with predominant mitral stenosis 2—5 years after operation. They considered the exercise test a valuable aid for predicting the risk and following-up the effect of operation. Chapman et al. (1960) deter mined the maximal oxygen intake during exercise on a motor-driven treadmill and found it to increase in all the seven patients they examined after valvulotomy

Many reports have been published of hemodynamic studies before and after commissurotomy Since Hellems et al. (1949) and Lagerlöf & Werkö (1949) introduced the technique with the catheter in wedge position, f recording pressure curves which in all essential respect re identical to the left atrial curve studies of this kind have been possible without catheterization of the left heart. The use of hydrodynamic principles and of attempts to elate diastolic flow and pressure gradient across the mitral ori fice to the rea f the orifice (Gorlin & Gor

PURPOSE OF THE INVESTIGATION

The purpose of the present investigation was to evaluate the effect of mitral commissurotomy in patients with mitral stenosis operated in a uniform way and by the same surgical team and examined by the same methods including right heart catheterization both pre- and postoperatively. Other purposes were to ascertain

I. In what degree this effect of operation was related to valvular anatomy before and after the split of the valve

II. How the different pre-postoperative differences in the results of different examinations were related to the functional state of the patient before and after operation evaluated from the history of daily activities.

III. What were the best methods for indication of the functional results of the operation.

IV. The relation between a calculated index of mitral area and hemodynamic and clinical findings at the postoperative examination in patients *investigated by both right* and left heart catheterization.

lin, 1951 Rodrigo 1953) have facilitated the analysis of the altered hemodynamics in mitral stenosis and have thus improved the prospects of studying the hemodynamic changes after commissurotomy

Most such studies have been carried out from a few weeks to one year after operation (Eliasch, 1952 Werkö et al., 1953 Ellis et al 1954, Wade et al., 1954, Wood, 1954 Ferrer et al., 1955 Dickens et al., 1957) The number of postoperative investigations with several years follow up and studies both at rest and during exercise by uniform methods is still quite small Donald et al. (1957) studied 28 patients before and after mitral valvulotomy with a follow-up period varying between 17 and 51 months. They found throughout a lower pressure in the pulmonary vessels after the operation and a marked reduction of the work of the right ventricle at rest, a diminished cardiac output at rest and a greatly reduced ventilation at rest and on exercise.

Further analyses of these results were made by Wade & Bishop (1962) with special regard to cardiac output at rest and during exercise. Lyons et al. (1959) examined 12 cases 8—30 months after mitral commissurotomy They found a lowering of the pressure in the pulmonary vessels, an increase of the resting cardiac output, a marked lowering of the pulmonary vascular resistance and in three patients signs of restenosis of the mitral valve.

Some postoperative hemodynamic studies have been made with the object of illustrating especially the postoperative condition of cases with increased pulmonary resistance (Merrill et al 1961) As in other similar studies, they found that the increased pulmonary resistance in cases of mitral stenosis is usually reversible, but that some increase often persists after valvulotomy

The problem of mitral restenosis has been illustrated in many studies (Soulié et al., 1957 Belcher 1959 Patterson & Marshall, 1959 Wilcken, 1960 Wilhelmsen et al., 1962) According to many authors the main cause of restenosis is incomplete division of the commissures at operation.

Table 2

Follow-up time (Mean follow-up time 34.8 ± 1.7 months)	Number of patients
0 — 1 years	3
1 — 2 years	9
2 — 3 years	21
3 — 4 years	15
4 — 5 years	11
5 — 6 years	1
Total umber of investigated pa ients	60

Tabl 3

	Sex			ε—
Age at operation, years	♂ + ♀	60	40.0	1 0
Age at operation, years	♂	18	38.7	2.2
Age operation, years	♀	42	40 6	1 1
Preoperative body weight, kg	♂	18	68.3	1 9
Preoperative body weight, kg	♀	42	57.2	1.5
Preoperative body length, cm	♂	18	175.0	2.1
Preoperati body length, cm	♀	42	162.5	1.3

years (f g 1 table 3) The mean body weight of the males was 68 3 ± 1 9 kg, of the females 57.2 ± 1 5 kg

The mean body length of the males was 175 0 ± 2 1 cm, of the females 162.5 ± 1 3 cm

S l ct d patie t bo ald not me t post p at xamination

Ten of the original 70 patients were not examined after operation. T of them had

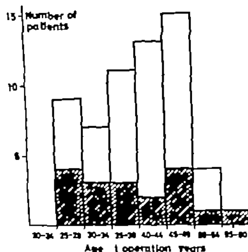


Fig. 1 Number of patients in different groups of age at time of heart operation. Blank columns denote number of females, and hatched columns denote males.

died, one 16 days after valvulotomy the other later during the follow-up period (table 1)

The remaining eight patients were not prevented from coming to postoperative examination by cardiac symptoms or signs, as will be seen from table 4 One patient (case 71) was being treated in another hospital at the time of the examination for thrombocytopenic purpura and another (case 65) was being treated for mammary tumor

Comments

Representativity of the material

Selectio ac ording to plac of residen

Most of the patients examined before commissurotomy at Karolinska Sjukhuset were admitted from county hospitals. The majority of patients living in Stockholm had been examined in other Stockholm hospital and were thus not included in the present material. Whether this method of selection

MATERIAL

The material consisted of patients with *mitral stenosis* on whom commissurotomy was performed at the Thoracic Clinic, Karolinska Sjukhuset. All patients investigated by right heart catheterization before operation at Karolinska Sjukhuset and operated during the period January 1957 to December 1960 were selected for the present study.

Indications for operation

The only important indication for commissurotomy in all patients was mitral valvular disease with pure or dominating stenosis. Commissurotomy was performed irrespective of age.

Number of patients

Althogether 70 patients were selected in this way. *Postoperative examinations* were performed on 60 of these patients at Karolinska Sjukhuset (table 1). The present material is composed of the latter patients. In 51 of these 60 the examination was made after the patients had been asked to come for postoperative examination. These investigations were performed during the period January 1962—January 1963. The other nine patients were examined at the hospital for other reasons. One patient was admitted to the hospital during the same period on account of a series of ventricular extrasystoles (case 27). Another patient (case 34) had been postoperatively examined at Karolinska Sjukhuset in 1960 eighteen months after the heart operation. Seven patients were admitted to the hospital on the following indications: heart failure (cases 55, 59 and 62), asthma cardiale (case 57), atrial fibrillation resistant to therapy (case 56), bacterial endocarditis (case 72), uncharacteristic changes in pulmonary X-ray (case 53).

Of these 7 patients 2 died during hospitalisation (cases 62, 72), one of them (case 62) with signs of congestive heart failure and the other (case 72) of sequelae to bacterial endocarditis.

Table 1

	Number of patients
Patients who could come for postoperative investigation (2 deaths)	60
Patients who could not come for postoperative investigation (2 deaths)	10
Total	0

Follow-up period

The mean period of follow-up of the examined patients was 34.8 months (table 2). 21 patients were examined from 2 to 3 years after the heart operation.

Sex and age distribution, body weight and body length

The material consisted of 42 females and 18 males, a proportion of females to males of 2.3:1. The mean age of the whole material at operation was 40.0 ± 1.0 years, males 38.7 ± 2.2 years and females 40.6 ± 1.1

Table 2

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4 — 5 years	11
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Total number of investigated patients	60

Table 3

	Sex		‾	ε
Age at operation, years	♂ + ♀	60	40.0	1.0
Age at operation, years	♂	18	38.7	2.2
Age at operation, years	♀	42	40.6	1.1
Preoperative body weight, kg	♂	18	68.3	1.9
Preoperative body weight, kg	♀	42	57.2	1.5
Preoperative body length, cm	♂	18	173.0	2.1
Preoperative body length, cm	♀	42	162.5	1.3

years (fig. 1, table 3). The mean body weight of the males was 68.3 ± 1.9 kg, of the females 57.2 ± 1.5 kg.

The mean body length of the males was 173.0 ± 2.1 cm, of the females 162.5 ± 1.3 cm.

Selected patients who could not come to postoperative examination

Ten of the original 70 patients were not examined after operation. Two of them had

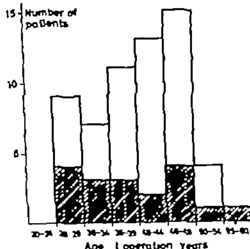


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Selection according to place of residence

Most of the patients examined before commissurotomy at Karolinska Sjukhuset were admitted from county hospitals. The majority of patients living in Stockholm had been examined in other Stockholm hospitals and were thus not included in the present material. Whether this method of selection

Table 4
Reasons why 10 patients did not come for postoperative investigation

Minors (cases, 67 68 69)	3
Other diseases than heart disease (cases 65 71)	2
Pregnancy (case 64)	1
Declined to come for investigation (case 70)	1
Medical papers not found until afterwards (case 66)	1
Deaths	2
Total	10

of the material implied also a selection as to character and degree of the valvular disease is difficult to decide.

Selection according to indications and contraindications for operation

If a dominating or pure mitral stenosis was diagnosed at the preoperative examination, the patient was operated upon unless a complicating severe disease of other kind was considered a contraindication. More important than this selection was probably the selection made at the county hospitals on account of age and other data.

Selection according to requirements for preoperative examination

The requirements for the selection of the present material, consisting in a complete preoperative examination, might possibly influence the representativity of the material.

Five patients were excluded from the material on account of incomplete preoperative examination. Two of them were foreigners who had been preoperatively examined in another country In another the preoperative heart catheterization was interrupted on account of asystole and no further catheterization was made. The two remaining patients, both with atrial fibrillation, were selected for commissurotomy in the absence of heart catheterization on account of arterial emboli in the anamnesis Four of the five patients had pure mitral stenosis. As these five excluded patients were not extremes either as to character of the valvular disease or as to age it does not seem probable that their exclusion would influence the representativity of the material to any major extent.

Selection on account of patients not coming to postoperative examination

Some data from the preoperative examinations of the eight females who did not come to postoperative examination are shown in table 5 In this group there was a higher frequency of sinus rhythm and a somewhat lower mean age than in the postoperatively examined patients. Preoperative findings, for instance heart volume, groupings according to the New York Heart Association (N.Y.H.A.) classification and findings at operation did not show any great differences from the examined patients in the present material. In questionnaires sent to these eight females they were asked about the daily activities of which they were capable at the time of the postoperative examinations. They were also asked to make comparisons between their present physical condition and their condition at the time immediately before the heart operation. From their answers to these questionnaires in respect of activities which resulted in breathlessness and of other symptoms and activities which could be performed without distress the patients were grouped according to the N.Y.H.A. classification. A comparison between these groups before and after operation

Tabl 5

Data / gbt patents be ould not come for post perative in stigations

Case No.	Age at opera-tion, years	Sex	Groups accor ding N.Y.H.A. class fication	Heart volume ml.	Rhy hm	Groups *)	Max.work in en sity performed kpm/min — pulse frequency	ding supine	Pulmo-nary artery mean pressure at rest mm Hg	Pulmo-nary mean wedge pressure t rest mm Hg	Cardiac output l/min a rest	Groups Fin-dings t ope-ration *)	After operation: Clinical condi-tion com-pared t preop. status*)	After operation: Groups accor ding to N.Y.H.A. classi-fication
64	30	♀	III	1090	S	A	400/170	—	27	[illegible]	5.2	1	Bet er	III
65	34	♀	II	1370	F	A	300/180	300/156	26	19	6.0	1	Status quo	II
66	47	♀	III	870	S	B	400/140	400/159	19	13	5.6	1	Much bet er	I
67	34	♀	III	805	S	A	600/168	400/140	23	19	7.1	2	Better	I
68	35	♀	I	960	S	B	400/148	—	17	13	4.8	2	Status quo	III
69	32	♀	II	850	S	A	450/161	—	33	26	6.3	1	Much better	I
70	54	♀	II	830	S	A	300/104	—	19	13	6.3	2	Better	II
71	39	♀	II	1210	S	A	209/60	200/156	64	34	4.2	1	Better	II

) The groups A, B, 1 2, are defined as in table 20. *) Patient own evaluation.

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Data f eight pat ts who auld not c me fo post perat ve estigations

	Before operation												After operation	
Case No.	Age at opera-tion, years	Sex	Groups accor ding N Y.H.A clas-sifica ion	Heart olume ml.	Rhythm	Groups [1]	Max.work intensity performed kpm/min — pulse f equency sit ing supine		Pulmo-nary artery me pressur at rest mm Hg	Pulmo-nary mean wedge pressure t rest mm Hg	Cardiac output l/min at rest	[1] G oups I ac ding t ope-ration	Clinical condi-tion com-pared t preop. st us [2]	Groups accor ding t N Y.H.A lassi fication
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IV

METHODS

Calibrated phonocardiography

Apparatus

All the phonocardiograms were recorded with the same type of apparatus, Mingograph Cardirex 42 with heart-sound amplifier type 42 A[1]. In this apparatus there are six high pass filters for dividing the frequency range of heart sounds into differen frequency bands. The second, fourth and sixth of he bands corresponding to nominal frequency of 25 100 and 400 c/s respectively were used in all examinations.

Frequency re po and amplification

The frequency response of the multiple filter ystem including microphone, amplifier and galvanometer is shown in fig. 2. The heart sounds recorded without amplification the urface of the chest decrease in intensity in such manner that the intensity is approximately in ersely proportional to the square of the frequency. This is compensated in the apparatus. The microphone was an electromagnetic air transmission ype.

Linearity

The apparatus was direct jet ink writer with linear response to about 500 c/ and time constant of about 2 sec. The paper was

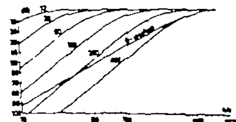


Fig. 2. Frequency response of the multiple filter system including microphone, amplifier and galvanometer.

[1]) Elema-Schönander AB.

dri en by synchronous motor. The sensiti vity of the electrocardiograph channels w adjusted to 10 mm mV before recording and the amplification of the entire phonocardiograph wa checked by placing the microphone on the calibrator of the apparatus. Synchronism was checked before every ecording. All the recordings were made at paper speed of 100 mm

The amplification of the heart sound amplifier can be increased stepwise. The step which produced clearly visible sounds and murmurs was chosen.

Referenc ecordi g microphone placement

The electrocardiogram, standard limb lead I, was alw ys used as reference recording. The chest areas were in all cases the right and the left second intercostal spaces at the sternal margin, the fourth lef intercostal space the sternal margin and he apex area. These recordings were made with the patient in supine position t rest. In most of the patients before operation and in all after operation, recordings were also made over other areas, for example on the left posterior axillary line in the fifth or sixth intercostal space, over the apex with the patient in the lef lateral position, the third left intercostal space at the sternal margin, and the first right intercostal space. When murmurs ere auscultated over the last two areas the ecordings were made both in sitting and in supine position. All the recordings were made fter meticulous auscultation over the entire precordium with the patient in supine, sitting and lef lateral position. Often, and in most of the examinations after operation, phonocardiographic recording was made also after exercise. After normal expiration the patient was asked to suspend respiration for the time necessary for the recording.

Calculations

Simultaneous interpretations are always made of patient pre- and postoperative

showed that six of the eight females belonged to group I or II after the follow-up period which is about the same proportion *as in the present material.*

Nor then, did this group of eight females seem to show any great difference in clinical condition after operation from the 60 patients who were examined postoperatively The two patients who died and were not investigated postoperatively will be considered in the section on mortality

Comparisons with other materials

The female-to-male ratio in the present material was similar to that in other materials of patients operated for mitral stenosis. This ratio is usually reported to be 3 1—4 1 (Hall, 1961 Otto 1964)

The female-to-male ratio in the present material with those patients added who could not come to examination after operation was 2.7 1

Mean age and age distribution were also very similar to those found in other materials. In the material studied by Otto (1964) the mean age was 37 years for women and 39 years for men in Hall's (1961) material the average age at operation was 38 7 years and in Wood's material (1954) 36.8 years

On the phonocardiogram variations in intensity of murmur in relation to time in the heart cycle within the same frequency band are less dependent on transmission and amplification than maximal intensity

Electrocardiography

Apparatus

All the electrocardiograms were recorded with four-channel direct writers (Mingograph Cardirex 4F[1]), the same type of apparatus as was used for the phonocardiographic recordings.

Frequency response, amplification, paper speed

The frequency response and time constant of the apparatus were as noted above. The recordings were made at paper speed of 50 mm/ with an ink jet of 40 mm length which gave maximum error of recorded amplitudes of 1 mm for ± 20 mm deflection.

Leads

The following leads were recorded in all cases: standard bipolar limb leads, unipolar augmented limb leads (VR, VL, VF) and bipolar chest leads with the negative electrode placed on the right arm, CR_2, CR_4, CR_5, CR. The indices indicate the same positions on the chest as the corresponding unipolar chest leads. In all patients one unipolar chest lead, V was also recorded. In standing position and during exercise on the bicycle ergometer the position of the indifferent electrode of the bipolar chest lead was changed to the forehead (chest-head lead, CH) to avoid muscle tremor interferences (Holmgren & Strandell, 1961). Plate electrodes and special electrode grease have been used in all cases.

Procedure

In all patients the electrocardiogram was recorded at rest with the patient in horizontal position and in almost all cases also in standing position after 8 minutes and during, immediately after and 4 minutes after work on the bicycle ergometer in sitting and in supine position.

[1]) Elema-Schonander AB.

At each recording the amplification was checked against test potential of 1 mV which was required to be recorded with 1 cm height. This calibration was simultaneous in all four channels so that the synchronism was checked at the same time.

Measurements for calculation of vectors

Mean vectors in frontal plane

The projection of the mean spatial P and QRS vectors on the frontal plane was determined by integration of simultaneously recorded potential curves form the same heart cycle in the extremity leads. The areas were calculated after measuring the base and height of the constructed triangles or trapezoums circumscribed by the QRS complexes and, sometimes, by the P waves and the reference base line. The P-R segment was used as reference base line. If in any one of the six leads the areas above and below the reference line were equal (equiphasic complexes), the lead was considered to form right angle with the frontal mean vector. If not, the net areas of the P waves and QRS complexes were plotted on the corresponding axes of the lead in one of the two three-axial reference figures for the frontal plane and the orientation of the mean frontal vector was determined in the usual way (Grant, 1957, Massie & Walsh, 1960).

Index of mean vector in the horizontal plane

To get measure of the orientation of the projection of the spatial vector in the horizontal plane the net areas in leads CR_1 and CR_2 were calculated in the same way as described above and the difference between them was used as an index of the mean vector in this plane. These CR leads were chosen because they were recorded in all patients whereas, with the exception of V_1, the unipolar chest leads and CR_1 were not.

Time relations

The duration of P wave, P—Q interval and QRS complex was measured on the standard bipolar limb leads at rest. The lead on which each of these durations was longest was used for the final measurement.

recordings before working up the data. It was usually possible to find heart cycles of the same length both pre- and postoperatively. The time relations between electrocardiograms and sounds, and between sounds, were always measured immediately after such heart cycles.

Comments on phonocardiographic methods

Errors

The evaluation of phonocardiographic findings is always to some extent dependent on the recorded amplitudes of sounds and murmurs. In different individuals the relation between the intensity of murmurs and sounds recorded by intracardiac methods and in the usual way outside the thorax is always different. This is true even if there is a compensation for frequency-dependent changes of intensity in the phonocardiograph and the amplification of the sound picked up by the microphone can be controlled by calibration. This relation between the intensity of murmurs and sounds recorded from inside the heart and from the thorax depends on the position of the heart on the sound transmission — which is dependent on the amount of air and of blood in the lungs — and on changes in the pleura. Th transmission is also dependent on the type of microphone and how it is applied to the chest du ing the recording.

If t were possible to eliminate the variations in sound transmission, t would be interesting to relate the intensity of sounds and murmurs in mitral stenosis to the results of other examinations, but using the usual phonocardiographic method such measurements of intensities would be too inaccurate. Nor would such a method be accurate for intraindividual comparisons n the present study as the sound transmission may ch ge considerably after thoracotomy.

Also the relating of intensity of sounds and murm rs to the intensity of the first and se cond so nd nvolves serious sources of error since changes in transmission may influence the intensity of sounds and m rmurs in different directions. The effect of thoracotomy on the transmissi n of sounds and m rmurs in this study could not be evaluated by th methods employed.

It is not possible however to exclude an evaluation of intensities totally because, when grouping the patients with regard to the existence or nonexistence of sounds and mur murs, transmission and amplification are often decisive for the result. In the present study an attempt was always made to record sounds and murmurs which were audible. But mu murs of small energy levels and within the frequency range 500—2000 c/s (Luisada & Di Bartolo, 1961) are sometimes difficult to record as the ear is more sensiti e to such mur murs than the phonocardiograph. Faint regur gitation m rmurs generated in the pulmonary and aortic region are examples.

The sound pressures of the audible heart murmurs are about 25—65 db (Butterworth et al., 1955) and the ear has its greatest sensitivity within this range for freq encies of about 100 c/s according to the Fletcher Munson curves. For sounds and murmurs of low and medium frequency the phonocardiograph is more sensitive than the ear. Murmurs of low ntensity and of high frequency were often found to have a higher amplitude after im provement of the transmission by change of the patient's body position and by recording after maximal expiration.

In the present study only clearly visible extra sounds or murmurs with the same time relations to the first and second sound in every heart cycle or n the case of irregular rhythm in most of the heart cycles, were included. Clearly visible sounds and murmurs were defined as recorded vibrations which because of their constant time relations and their constant configuration on the recordings, could safely be d fferentiated from artifacts.

Time relations between the different sounds are less dependent on ariations in transmission and in amplification. The time from the Q f the QRS complex to the f rst high vibra tions of the first sound Q—1 nterval, is versely correlated to the length of the preceding heart cycle and the time from the f rst distinct vibrat ons of the second sound to he opening snap 2—OS time is directly cor related to the preceding heart cycle (Messer et al., 1951). As mentioned above, these t m r lations were both pre- and postoperat vely measured after heart cycles of th same length.

On the phonocardiogram variations in intensity of murmur in relation to time in the heart cycle within the same frequency band are less dependent on transmission and amplification than maximal intensity.

At each recording the amplification was checked against test potential of 1 mV which was required to be recorded with 1 cm height. This calibration was simultaneous in all four channels so that the synchronism was checked at the same time.

Electrocardiography

Apparatus

All the electrocardiograms were recorded with four-channel direct writers (Mingograph Cardirex 42[1]), the same type of apparatus as was used for the phonocardiographic recordings.

Frequency response, amplification, paper speed

The frequency response and time constant of the apparatus were as noted above. The recordings were made at paper speed of 50 mm/s with an ink jet of 40 mm length which gave maximum error of recorded amplitudes of 1 mm for ± 20 mm deflection.

Leads

The following leads were recorded in all cases: standard bipolar limb leads, unipolar augmented limb leads (VR, VL, VF) and bipolar chest leads with the negative electrode placed on the right arm, CR_2, CR_4, CR_5, CR_7. The indices indicate the same positions on the chest as the corresponding unipolar chest leads. In all patients one unipolar chest lead, V_1, was also recorded. In standing position and during exercise on the bicycle ergometer the position of the indifferent electrode of the bipolar chest lead was changed to the forehead (chest-head lead, CH) to avoid muscle tremor interferences (Holmgren & Strandell, 1961). Plate electrodes and special electrode grease have been used in all cases.

Procedure

In all patients the electrocardiogram was recorded at rest with the patient in horizontal position and in almost all cases also in standing position after 8 minutes and during, immediately after and 4 minutes after work on the bicycle ergometer in sitting and in supine position.

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[1]) Elema Schönander AB.

Measurements for calculation of vectors

Mean vectors in frontal plane

The projection of the mean spatial P and QRS vectors on the frontal plane was determined by integration of simultaneously recorded potential curves form the same heart cycle in the extremity leads. The areas were calculated after measuring the base and height of the constructed triangles or trapezoims circumscribed by the QRS complexes and, sometimes, by the P waves and the reference base line. The P R segment was used as reference base line. If in any one of the six leads the areas above and below the reference line were equal (equiphasic complexes), the lead was considered to form a right angle with the frontal mean vector. If not, the net areas of the P waves and QRS complexes were plotted on the corresponding axes of the leads in one of the two three-axial reference figures for the frontal plane and the orientation of the mean frontal vector was determined in the usual way (Grant, 1957, Mazzia & Walsh, 1960).

Index of mean vector in the horizontal plane

To get a measure of the orientation of the projection of the spatial vector in the horizontal plane the net areas in leads CR_7 and CR_2 were calculated in the same way as described above and the difference between them was used as an index of the mean vector in this plane. These CR leads were chosen because they were recorded in all patients whereas, with the exception of V_1, the unipolar chest leads and CR_1 were not.

Time relations

The duration of P wave, P—Q interval and QRS complex was measured on the standard bipolar limb leads at rest. The lead on which each of these durations was longest was used for the final measurement.

Comments on electrocardiographic methods

Erro s

When the projection of the spatial instanta neous P and QRS vector on the frontal plane is small, the errors in measurement must be great. When P and/or the QRS loop in the frontal plane is related in such a way to the isoelectric point that equiphasic complexes are inscribed in several leads, a mean vector is difficult or impossible to calculate in the way described above. If by inspection it was diffi cult to decide whether the positive or negative area of the P waves and QRS complexes was largest in two or more leads, no attempt was made to calculate a mean vector

The magn tude of the mean vector depends on many factors such as the manner of appli cation of the electrodes, the moisture of the skin and the existence of edema. In the present study the magn tude of the mean vector was not reported.

The direction of the mean vector in the frontal plane is essentially independent of small changes in placement of the electrodes on accou t of their relatively large distances from the heart. The direction of the mean vectors is influenced by body position. All the measurements were made on electrocardiograms ecorded on patients n supine position Only some of the patients had more than o e pillow under the head during recording

It may be assumed that the calculations of the index of the mean QRS vector in the horisontal plane on the b polar precordial lead are less accurate than those of frontal mean vector The net areas of the QRS compl es in these leads change after small alterat ons n the positions of the electrodes. S ce th angles of the axes of the chest lead in relation to the fro tal plan a not the same d fferent i dividuals owing to the largeness and configuration of the th ra the direction of the mean ectors the horison tal plane cannot be calculated in th way with great accuracy In the present study t may be assumed that the pre-postoperati e cha ges in the net areas of the QRS complexes CR_7 nd CR were essentially uni fluenced by any of hese errors as they were t nd idual d fferences.

Reproduci b lity

The reproducibility of these measurements was calculated from 15 duplicated measurements, each of which was made on recordings from the same patient at an interval of some days. The standard deviation of the single estimation of the difference in net area between CR_7 and CR_2, thus including both biological and technical errors, was 4 4 mm² on the elec trocardiographic paper corresponding to 8.8 10⁻ volt seconds with a coefficient of aria tion of 18.8 %.

S T Intervals and T es

As 49 of the patients in the present material had been treated with digitalis and some also with quinidine, and as some recei ed this therapy only before and others only after the operation, a uniform evaluation of pre- and postoperati e S-T intervals and T waves was not possible on account of the lack of a method for d fferentiating between the effect of pathological changes in the heart and the effect of the therapy

Exercise test on bicycle ergometer

Apparatus

The same type of bicycle ergometer[1]) (Holmgren & Mattsson, 1954) was sed in all the exercise tests. It is an electrodynamically braked ergometer with pproxim tely constant load at pedall g rates varyi g between 50—70 c/min.

Cal b ation

During the period of examination the bicycles were calibrated t intervals of from 9 mo ths to 2 y ars. The gre test changes in dial setting—work load rati between calibrations was found at the sm ll load corresponding to the l west part f the calibration curve where u ha ges in d al setti g corresponded to the smallest hanges in load. W th the r nge from 100 to 300 kpm mi th load sometimes ha ged duri g these inter ls by up to 30 of the earlier al e t the sam d'al setting b t loads bove 300 kpm m n the changes were ever gre ter tha 10 of the ea lier al e.

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[1]) Elema Schönande AB.

Procedure

When the clinical condition permitted, the patients worked both in sitting and in supine position. By using the ratemeter the patients were able to pedal at an almost constant rate of 60 cycles/min.

Exercise test in sitting position

After recording of the electrocardiogram at rest and after 8 minutes in standing position the patient lay down for some minutes and then returned to the ergometer for a further recording before exercise started. The exercise test was performed according to the method of Sjöstrand (Sjöstrand, 1947, 1960, Wahlund, 1948).

When the physical working capacity of the patients was estimated to be only moderately decreased, successive multiples of 200 kpm/min for women and 300 kpm/min for men were chosen as work intensities. When the capacity was estimated to be low, multiples of a smaller load, 100 or 150 kpm/min, were chosen.

Exercise test in supine position

The exercise test in supine position was performed on the same principles as in sitting position. The reason for testing in the supine position was, among other things, to learn what work intensity the patient could achieve during heart catheterization. The patient exercised on the catheterization table with the ergometer clamped to the table and the feet fastened to the pedals of the ergometer. The patient pedalled around an axis 15—25 cm above the horizontal plane at the level of the patient's back and with a stroke radius of 17—18 cm. The patient held onto two handles fastened to the catheterization table. Some patients had more than one pillow under the head during exercise.

Reasons for interruption of the test

The patient performed the exercise test until marked symptoms appeared, usually marked dyspnea or intense general tiredness. In some cases the test was stopped on account of a high heart rate, 170 beats/min or more, but in these cases the dyspnea or tiredness was so marked that the patient was not expected to be able to work at a higher load. In some cases a high frequency of extrasystoles caused the test to be interrupted.

Evaluation of maximal work intensity (W_{max})

The greatest work intensity which the patient was able to sustain during six minutes was regarded as the maximal work intensity. When a patient's symptoms prevented work at a load for as much as 6 minutes, the highest work intensity (W_{max}) was estimated to be the next lower load at which the patient was able to work for six minutes (W_6) and to this load was added $\frac{n \cdot W_d}{6}$ kpm/min, where n is the number of minutes during which the patient exercised at the highest work intensity and W_d the difference between the loads in kpm/min. Thus $W_{max} = W_6 + \frac{n \cdot W_d}{6}$.

Work intensity-pulse rate relation $\frac{W}{\Delta f}$

In order to get a measure of the relation between work intensity and heart rate in cases of sinus rhythm, a ratio was used in which the numerator consisted of the work intensity sustained during 6 minutes (W_6) and the denominator of the increase in heart rate from rest in sitting position to 6 minutes work at the same intensity (Δf). Thus the relation was expressed as $\frac{W}{\Delta f}$ i.e. kpm/min pulse beat.

Whenever possible the work intensities which, both before and after the heart operation, caused about the same heart rates within the range 120—150 beats/min were chosen as numerators. Tests with heart rates below 100 beats/min at the highest load sustained during 6 minutes were omitted.

Calculation of heart rate

In exercise tests in sitting position the heart rate at rest was counted on electrocardiograms recorded at a paper speed of 50 mm/s with the patient sitting on the ergometer immediately before the start of work. In these recordings the heart rate was usually calculated from three heart cycles. In all other countings of heart

rates electrocardiograms were recorded every second minute for 10—15 seconds at sinus rhythm (paper speed 25 mm/s) and for 30 seconds at atrial fibrillation (10 mm/s) All heart rates were thus calculated from electrocardiograms and no values were intra or extra polated.

Comments on methods of exercise test

Errors

The changes in dial setting—load ratio between calibrations of the ergometers were rather large at the smallest loads in relative but not in absolute values. No error of importance in the values of W_{max} and $\frac{W_6}{\Delta t}$ of the different groups of patients was introduced by these changes, as the mean values of these work intensities corresponded to parts of the calibration curves where the changes between calibrations were small. Furthermore there were no systematic changes in dial setting—load ratios.

R producibil ty of W_{max}

The error in evaluating the maximal work intensity that a patient can attain depends among other things on the will of the patient and on the evaluation of the patient s symptoms during the test. The rep oducibility of the assessment of maximal work intensity w s calculated in 13 of the originally selected 70 patients, two of whom (cases 70 and 71) belo ged to the group of ten patients not in vestigated after the heart operation. Four of the 13 pat ents had atrial fibrill tion and the remainder sinus hythm

These calculations were based on all func tional test sitting position which were performed twice in two months. The reasons for performing the test twice were in some cases the desire to correlate l es of heart volume and blood volume to simultaneous exercise tests, in others th fact th t test had been performed some weeks before the patient was admitted to hospital and new test was made after admission. The only test performed twic w thin two months which was not used for these calcul t ons was that on patient no. 2 who showed such a great discrepancy between symptoms and sign that the examining doctor wished to test him at varying loads unknown to the patient. By this arrangement it was possible to persuade the patient to work at much heavier loads on the seco d occasion. The range of the time differences between the two tests in all 13 patients was 1—61 days with a mean value of 13 days. The mean d fference in maximal work intensity between the first and second test was — 38.6 kpm ± 16.0 ($0.0 < P < 0.05$). If the difference was regarded as non significant, the standard deviation of a single estimation was 47.7 kpm/m in all the 26 measurements, corresponding to a coeff cient of variation of 13.8 %. The mean value of the loads was 345 kpm in these duplicate tests with a range of 100—700 kpm/min. The thirteen d plicate tests were performed on different bicycles in nine cases.

The calculation described abo e and used when the patients had worked less than 6 minutes at th highest load is approximate as only one point is known on the individual curve which describes the dependence of maxi mal work ntensity on max mal performance time and, furthermore a this relation not linear at least in normal individ al (Grosse et al 1937 Tornvall 1963) The error introduced by this calculation seems to be small in nor mal i d viduals (Strandell, 1964). I that study the measured maximal oxygen uptake agreed very well with the value calcul ted in this way Thi ma ner of calculating ma mal work intensity was preferred i the present study as repeated maximal work tests pa tients in different clinical condition were not regarded as advisable A yway the m imal work intensity calculated in this wa seem to be a more accurate value than th lowe work intensity sustained d ring six minutes.

St ady tate

The circulatory adaptation to work is slow or incomplete n many patients with mpaired h art function. Jongbloed et al (1957) showed by continuous record g of oxygen consumption th t many patients with mitral stenosi b cycling at a load of 60 watts (=367 kpm min) for 8 m utes reached steady state with reg d to oxygen transport either very la e duri g the period or ot t all. I a study by Donald et l. (1954) there wa mean ncre se of 15

beats/min in heart ate from the second to the fourth or fifth minute of work with *an oxygen* uptake ranging from 300 to 600 cc min in 8 patients with mitral stenosis and with sinus rhythm.

In 20 patients with sinus rhythm randomly selected in the present material, working at the highest load for 6 minutes, the mean increase in heart rate from he second to the sixth minute was 9.5 beats min with range of 1—13 beats/min. No ttempt was made to separa patients working in circulatory steady state from those who were not.

W ork rate sity — pulse rate atre

Often pronounced dyspnea forces patient with mitral stenosis stop working at ther low pulse rates. I such cases an evaluation of the physical working capacity from the work intensity — pulse ratio is not possible. Also in these patients, however the ratio $\frac{W}{\Delta f}$ provides some idea of the circula ory adapta tion

A in the present study the lowest load was chosen with regard to the clinical condition of the patient, multiples of the smallest load were not the same in different patients. I was thus not possible to compare pulse rates in different patients at the same load. In healthy individuals the mechanical efficiency increases successively up about 300 kpm/min and is hen rather constan at higher loads on the bicycle ergometer (Åstrand, 1960). As the exercise test in the present stud was made at arying and in several cases rather low work intensities, there was probably ariation in mechanical efficiency between the patients According to the comments above, it is also reasonable to assume differen degrees of or lack of circulatory steady state, and decrease of stroke volume is characteristic finding during exercise in tight mitral stenosis. A linear extra- or intrapolation of pulse rates to some value common to all patients in order to assess physical working capacity was thus not made.

Furthermore, many patients were not able to work at more than one load, so it was not possible to study how heart rate varied with work intensity

For these reasons he relation $\frac{W}{\Delta f}$ was studied about the same heart rate before and after heart operation in the same individual.

Reproducibility f $\frac{W}{\Delta f}$

The reproducibility of work intensity—pulse te ratio is very good in normal individuals working on bicycle ergometers, as was shown i duplicate ests by Borg & Dahlström (1962). They also showed that the reproducibility was best at higher work intensities. The eproducibility of $\frac{W}{\Delta f}$ In he present material was studied in the same duplicate tests as described abov but with the patients with atrial fibrillation eliminated. In these eigh duplicat ests the mean alue of $\frac{W}{\Delta f}$ was 6.0 ± 0.9 *kpm/min pulse beat with standard deviation* of single estimation of 1.9 (coefficien of variation 22.2). The mean load was 335 kpm/min and the mean value of heart rates 142.8 min.

There were no significant systematic differences between $\frac{W_f}{\Delta f}$ in the first and in the second test the work in ensities used for calculations of the ratios.

As some of these duplica tests were performed at intervals of up to tw months it may be assumed that alterations in the condition of some pa ients on account of change in physical acti ity or in medical treatmen influenced these figures of reproducibility markedly

Heart volume estimation

Chest X ray examinations at right angles were made with the patient in prone position and with the central ray angled 30° caudally in the anterior view (Larsson & Kjellberg, 1948 Kjellberg et al., 1949). The calculation of heart olume from X-ray pictures was done by the method of Larsson & Kjellberg (1948). In these calculations based on the formula of Rohrer (1916/17) and Kahlstorf (1937) there is an empirically found factor which is func tion of the square of the greatest diameter

rates electrocardiograms were recorded every second minute for 10—15 seconds at sinus rhythm (paper speed 25 mm/s) and for 30 seconds at atrial fibrillation (10 mm/s). All heart rates were thus calculated from electrocardiograms and no values were intra- or extrapolated.

Comments on methods of exercise test

Errors

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These calculation were based on all f c tional tests in sitti g posit on which were performed twice in two months. The reasons for performing the test tw ce we in some cases the desire to correlate *alues of heart olume* and blood ol me to sim ltaneous exercise *tests, i others the* fact that a test had been pe formed some weeks before the patient wa adm tted to hosp tal nd a new test was mad after adm sion. The only test performed twice with n two month which was not used for these *calcul tion* wa that on *pati nt no. 2* who showed such a gre t d screpancy between *symptom and sign that* the exam ning doct r wished to test him at varying loads unknown to the patient. *By this arrangement it was pos*sible to persuade the patient to work at much heavier loads on the second occasion. The range of the time differences between the two tests in all 13 patients was 1—61 days with a mean val e of 13 days. The mean d fference in maximal work intensity between the first and second test was — 38.6 kpm ± 16.0 ($0.02 < P < 0.05$). If the difference was regarded *as non-significant, the standard dev ation of a* single estimation was 47.7 kpm/min in all the 26 measurements, corresponding to a coefficient of ariation of 13.8 /o. The mean alue of the loads was 345 kpm in these duplicate tests with a range of 100—700 kpm/min. The thirteen duplicate tests were performed on d fferent b cycles in nine cases.

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St ady state

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carbon monoxide[1]) with thoroughly calibrated syringe.

The relation between the degree of saturation of hemoglobin with carbon monoxide and oxygen, respectively $\frac{CO\ Hb}{O_2\ Hb}$ is function of the partial pressures of CO and of O in blood and this function is expressed by Haldane equation, $\frac{CO\ Hb}{O_2\ Hb} = M \frac{pCO}{pO_2}$ where M is constant. After period of about 22 minutes it has been shown that equilibrium is reached in CO passage between the alveoli and the capillary blood in patients without lung disease and with the concentrations of CO used. As rebreathing period of 30 minutes is employed, there is margin for increased equilibration time. In the calculations the partial pressures of CO and O_2 in the alveoli are used since they are assumed be approximately the same as in the end capillary blood. Carlsten et al. (1954) found empirically that the most representative value of the constant M was 245 corresponding to 231 after change in the corrections of alveolar gas pressures (Wiklander 1956), and this also was used in the present material.

The amount of added carbon monoxide and the concentrations of this gas in the two gas samples and the volume of the whole system give the figures for calculation of the amount of carbon monoxide remaining in the system and taken up by the blood in the lungs. The olume of the closed system includes, in addition to the volume of the rebreathing apparatus, an assumed volume of dead space of the airways and of the volumes of the lungs in certain respiratory phase. Fairly large changes in these assumed values have very little influence on the final result of the calculations. After calculation of the partial pressure of CO and O_2 in the alveoli the concentration of CO in hemoglobin before and after injection of CO in the system can be estimated by Haldane's equation.

The uptake of CO divided by the increase of CO concentration in hemoglobin gives the total amount of hemoglobin. A simplified formula for the calculations (table 6) worked out

Table 6

Approximate formula for calculation of total hemoglobin (THb g)

$$THb = \frac{2.60 \cdot V \cdot F_{ICO} \cdot F_{O_2} \cdot K_{P_B} \cdot K_T}{1.000 \cdot (F_{CO_{II}} - F_{CO_I})}$$

V = volume of concentrated CO gas supplied to the = 18.72 ml ATP

F_{ICO} = fraction of CO in the gas supplied to the system = 0.995

F_{O_2} fraction of O_2 in the second sampling rubber bag

F_{CO_I} = fraction of CO in the first sampling rubber bag, before the supply of CO

$F_{CO_{II}}$ = fraction of CO in the second sampling rubber bag, 30 min after supply of CO

K_{P_B} = correction factor for the barometric pressure. The slope of the factor on P_B was positive. A 760 mm Hg the factor was 1.000, for change of ± 10 mm Hg it changed by ± 0.0138.

K_T = correction factor for the room temperature. The slope of the factor on emperature was negative. A 21.6 C the factor was 1.000, for change of ± 1 C it changed by ± 0.0030.

by Linderholm (unpublished observations) and modified by Strandell (1964) was used in this material. The standard deviation of the differences between those calculated with the complete formula and with the simplified one in 100 randomly selected patients investigated in this laboratory was ± 2 of the mean value of the amount of hemoglobin (Strandell 1964).

Apparatus

In the apparatus used the determination of CO concentration in the gas samples is made by recording the increase of temperature caused by oxidation of CO over catalyst, Hopcalite (Sjöstrand, 1948). The type of apparatus was SL—2, AB Seller, in which the changes in temperature are measured by thermistors (Linderholm & Sjöstrand, 1956). The rebreathing apparatus was of type Aga M E 1550 (Linderholm, 1957).

[1]) Matheson Corp Ill., through Kebo, Stockholm.

of the heart in the lateral view divided by the product of the greatest longitudinal diameter and the greatest diameter perpendicular to it on the postero-anterior projection. All diameters were corrected for magnification. The exposures were made irrespectively of the phases of the cardiac cycle and after inspiration. The radiograms of each patient were not taken simultaneously. The perpendicular distance between the film and the focus was 125 cm in both projections. Contrast filling of the esophagus made the drawing of heart contours easier. They were drawn, redrawn or checked by me, and all calculation of volumes were checked or made by the same technical assistant.

Comments on methods of heart volume estimation

Reproducibility of contour drawings

The error in drawing the contours of the heart was analysed in a material of mitral stenosis by Linderholm & Strandell (1958). The female-male ratio and the incidence of patients with sinus rhythm were the same as in the present material. The authors found a coefficient of variation of 3—4.5 % in different groups of dominating mitral stenosis computed from duplicate determinations from two drawings made by the same examiners and on the same picture in 30 patients. The radiological technique and the methods of calculation were the same as in the present study. This source of error was not analysed in the present study.

The decrease of heart volume in standing position compared with the volume in prone position had been observed already by Moritz (1904). Larsson & Kjellberg (1948) showed that this decrease of heart volume was linearly correlated to the heart rate in standing position in normal individuals and that the decrease was about 15 % at a pulse rate in standing position of 120/min. They found also that in prone position the heart volume did not change with varying pulse rates up to about 120/min. In the study of Linderholm & Strandell (1958) there was no statistically significant difference between heart volumes in standing and in prone position in the patients with mitral stenosis. The authors supposed that this could be explained by a smaller orthostatic redistribution of the blood volume than in other groups of patients. In the present material no systematic comparison was made between heart volumes in prone and in erect position.

Accuracy

According to Kjellberg et al. (1951), from the theoretical point of view the oblique projection in the anterior view does not materially change the accuracy of the method. Heilborn & Strandell (1962) found in model experiments, however, that by angulation of the tube the volume of a vertical heart will be overestimated by 13 % and they considered that the mean projection error of fairly normal hearts was of the order of 5 %. Kjellberg et al. (1951) showed that in individuals without heart disease heart volumes calculated as described above from exposures made both in diastole and in systole in the same individual did not significantly differ. Nor did they do so if the exposures were made during slow superficial breathing or immediately after a deep inspiration.

Reproducibility

In duplicate estimations of heart volume Kjellberg et al. (1951) found a standard error of a single determination, expressed as per cent of the mean, of 3—4 % in different groups. In a study by Holmgren & Ovenfors (1960) with triplicate determinations the corresponding figure was 4.1 %. In the latter study the exposures in the two planes were made simultaneously and always in the same respiratory and cardiac phase.

Estimation of total amount of hemoglobin

Principles of estimation

The total amount of hemoglobin was estimated by the method of Sjöstrand (1948). According to this method the patient breathes almost pure oxygen in a closed system in which carbon dioxide is absorbed continuously. Gas samples from the system are taken before and after injection of usually 20 ml 99.5 %

Errors

Different sources of error of the alveolar CO method were discussed by Sjöstrand (1948), Carlsten et al. (1954), Dahlström (1955) and Wiklander (1956). The error of the method is increased in patients with high initial CO concentration in hemoglobin (Sjöstrand 1948). In the present material the mean value of he concentration was 0.93 %, slightly above the normal variation in individuals who do not smoke. The error caused by leakage into the closed system was diminished by checking the oxygen content in the system. The error was also diminished by duplicate determinations. A third determination was usually made if the difference between the values of total hemoglobin was greater than 10 of he lowest value.

Reproducibility

The reproducibility of the alveolar CO method was estimated by Wiklander (1956) in 92 duplicate determinations at intervals of 1—7 days. The standard deviation of single estimation was 14 g corresponding to coefficient of variation of 2.8 In 39 duplicate estimations made at the postoperative investigation in the present material the standard deviation of single estimation was 25 g and the coefficient of variation 4.1 %, corresponding to 2.9 for duplicate estimation. These figures were consistent with those found in earlier investigations (Sjostrand, 1948, 1953 Astrand, 1952, Hallberg, 1955). The coefficient of variation in estimations of blood volume and of hemoglobin concentration in the same number of duplicate tests was 4.1 and 2.7 respectively.

The red cell volume estimated by CO methods reported to be 12—16 larger than the same volume estimated by methods using other red cell labels (P^{32} Cr^{51} Fe^{55} Fe^{59}) in duplicate determinations (Nomof et al., 1954 Gregersen & Rawson 1959). Most authors explain this difference in uptake of carbon monoxide by myoglobin and by noncirculating hemoglobin (Gregersen & Rawson 1959). They did not employ the method of Sjostrand. Sjostrand' method does not make use of absolute values of CO concentration in hemoglobin but of the increase in concentration after addition of carbon monoxide. This means that the CO concentration in hemoglobin before addition of CO is determined. The oxygen content of the arterial blood is practically unchanged due to the low CO concentration in hemoglobin after the exposure and to the increase of the physically dissolved oxygen (about 100 oxygen in the inspired air). This difference in method is of importance, as stressed by Dahlström (1955) and by Sjöstrand (1962). In the calculation of total hemoglobin in the present material there is correction factor of 0.95 for uptake of carbon monoxide by myoglobin and by noncirculating hemoglobin.

A low diffusion capacity and changed relation between ventilation and blood flow may increase the rebreathing period before steady state occurs. Many studies have failed to disclose any significant disturbance in diffusing capacity in cases of mitral stenosis (Driscoll et al., 1963), or have shown such decrease only in the more severely affected cases (Riley et al., 1956, Forster 1957). An increased intrapulmonary physiological shunt is usual finding in cases of mitral stenosis. To judge from the values of arterial oxygen saturation (see later chapter) significant lengthening of the calibration period caused by intrapulmonary shunts does not seem to be an important error in the present material.

Estimation of oxygen consumption

Procedure

The basal metabolic rate was determined according to the Douglas method (Douglas, 1911) and also to the closed circuit method with pure oxygen in the spirometer (Krogh, 1923).

The investigation was performed early in the morning after the fasting patient had been transported to the investigation in his bed. Duplicate determinations were made by both methods. Low resistance valves were used in the Douglas method, and the breathing periods lasted 10 minutes.

Collection and analysis of expiratory gas

Almost all gas samples from the Douglas bags were analysed with Haldane apparatus. During short period some samples were ana-

Procedure

The total duration of the examination was about 50 minutes, during which the patient was reclined on a couch During the first 5 minutes the patient inspired from the system and expired into the air thus eliminating nitrogen from the airways. Fifteen minutes after the begin ng of the rebreathing period the f rst gas sample was taken. Carbon mon oxide was then injected into the system and 30 minutes later the second sample was taken. The samples were taken by disconnecting rubber bags from the closed system with a threeway stopcock.

R bber bags in the system were refilled with adequate amounts of oxygen at suitable inter vals during the investigation. The oxygen con centrations in both samples were determ ned in a Beckman oxygen analyser model C, in order to disclose any leakage nto the system If the alue of oxygen concentration in these samples was lower than 90 /, the investiga tion was started again fter checking of the system and of the fit of the mouthpiece In every patient dupl cate determination were made, w th the two determinations usually on consecuti e days.

Cal brat on

Carbon monoxide[1]) of approximately 0 05 and 0 010 / was used for calibration The con entration n a new tube was always determined by relating to the concentration of gas in the old tube with suc ess e analysis in on of the Hopcalite apparatuses. The absolute alues of the gases were formerly obtained by calibrating aga nst p re carbon monoxide (Lan derholm & Sjöstrand, 1956). Since June 1962 this cal bration has been done by another tech n que gi ing systematically lower alues of the amount of hemoglob n. This d fference s tested in healthy ndi iduals. All the values of total hemoglobin and of blood olume in this material before that time were corrected to this new absolute stand rd. The correction fac t r is 0 91

The Hopcalit pparatus was calibrated daily by analysing two test gases and checking the relation between the corresponding values. The linearity of the apparatus was checked frequently In every determination the test gases were used as reference. The oxygen[1]) was free from carbon monoxide.

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[1]) AGA Stockholm-Lidingö.

Estimation of blood volume

Blood volume was calculated from total amount of hemoglobin and from hemoglobin concentration. The latter was estimated from spectrophotometrical analysis of hemolysed finger blood and the extinction was read at 540 mμ (Sunderman et al. 1953). The calculated blood vol mes were corrected for a erage hematocrite. A value of 0.91 was used for the quotient between average hematocrite and enous hematocrite

Comments on methods for estimation of total amount of hemoglobin and blood volume

Comparis with ad oacti e t acer method

In study at this laboratory (Ekelund 196) the blood volume calculated from total amou t of hemoglobin measured by the alveolar CO method and from hemoglobin concentration was compared w th blood olume measured by tracer technique. The l tter measurement was made by a semia tomatic method (Williams & Fine 1961) using J^{131}-tagged serum albumin and a time of mixing of 10 minutes. A high-speed ce trifuge (10,000 p.m.) was used for hem tocrite determination and no correc tion was m de for trapped plasma. All the values were corrected for average hem tocrite The mean alue of plasma olume determined in 26 healthy men by the J^{131} method that study was 50.5 ml ± 1 1 ml/kg body weight and th mean quotient between the alues of blood olume determined with J^{131} and with CO respectively was not significantly different f om nity

In 21 patients in the present ma erial the blood volume was determined by both methods, as in the study bove, and with th same correction for average hematocrite. In th present study as well the mean quotient did not signif cantly differ from unity (= 1 031 ± 0.017)

Error

Different sources of error of the alveolar CO method were discussed by Sjöstrand (1948), Carlsten et al. (1954), Dahlström (1955) and Wiklander (1956). The error of the method is increased in patients with high initial CO concentration in hemoglobin (Sjöstrand 1948). I the present material the mean value of the concentration was 0.93 %, slightly above the normal variation in individuals who do not smoke. The error caused by leakage int the closed system was diminished by checking the oxygen conten in the system. The error was also diminished by duplicat determinations. A third determination was usually made if the difference between he alues of total hemoglobin was greater than 10 of the lowest alue.

Reproducibility

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Collection and analysis of expiratory gas

Almost all gas samples from the Douglas bags wer analysed with Haldane apparatus. During short period some samples were ana-

lysed with Scholander apparatus (Scholander 1947). The same techniques of collection and analysis of the expiratory gases described here were used in the determination of cardiac out put in the present material. The gas volumes were measured by dry or by wet gas meters in all the preoperative examinations. Samples were taken and volume measurements made shortly after the collecting periods. The bags were emptied by folding. The dry gas meters were calibrated regularly against wet gas meters and the latter against spirometers. Al most all postoperative determinations of gas volumes were made in an apparatus con structed by Stern (unpublished method), con s sting of two communicating tanks at different levels and with the upper one open to the air After connecting the Douglas bag to the top of the lower tank water is pumped from this tank up to the other and the bag is emptied by the induced underpressure in the lower tank. When the bag is completely emptied, as indicated by negative pressure in the lower tank, the valve of the bag is closed. Atmospheric pressure in the lower tank is then obtained, by allowing some water to return to it. The change of level of the water in the lower tank indicates the volume of gas in the Douglas bag

In the analysis with Krogh apparatus two curves were accepted only if they were regular with a difference between the tangents of the slopes of the curves of not more than about 4 %. Otherwise a new rebreathing period of 5 minutes was recorded. Outward leaks were checked by loading the bell both with and without the patient s a rways ncluded n the system. Temperature n the sp rometer and barometric pressure were read during ch exa mination. The curve with the slightest slope was used for the calculat on of oxygen uptake

The volume of oxygen onsumption ex pressed in volume of dry gas t standard tem perat re and pressu (STPD) was elated to predicted al es from tables of Harris & Benedict (1919)

Comments

Errors of Dougla thod

One of the n so rces of rror in estimating oxygen co sumption by the Douglas bag technique is the permeability of the bag to the respiratory gases on account of solution of gases in the rubber layer of the bag and the diffusion of the gases through the rubber (Balchum et al., 1953 Shephard, 1955). The change in concentrations of the respiratory gases in the bag is approximately linearly related to time. By sampling and measuring the volume of the bags shortly after the investiga tions, as was done in the present study this source of error may be disregarded.

The gas meters, both dry and wet, were operated at rates evaluated from calibrations.

As in the wet gas meter one source of error in volume measurements with the tank is the absorption of carbon dioxide in water As the tank was used daily and the water in t was not changed, the partial pressure of CO_2 in the water did not probably deviate much from that of expired air Thus this source of error seems to be negligible

Reproducibility

The standar error of a single estimation in 17 duplicate estimations of oxygen consumption by the Douglas bag technique in the first 17 postoperativ vestigations was 3 9 ml corresponding to coefficient of variation of 1.5 %. This figure thus includes the error both n vol me measurement and in determination of oxygen concentration. There was no sy stem atic d fference between the values of the first and the second period of examination.

Cl sed circ t method

Harmon (1953) found in an analysis of the sources of variation in determination f oxygen consumption by the closed circuit method standard error of estimate of 7 9 ml m min In a comparison between the open and the closed circui methods in randomly selected patients Fowler et L (1957) found o sy tem atic d fference between the two methods, and the tandard deviation of the d fferences between the methods expressed in percent f predicted values was about 7 %. Some of h most important sources of errors he losed circuit method (Fowler et al., 1957) were probably diminished in the present study by regular checks for leakage, and the fact th all investig tions were mad only by specialized nurses.

Reproducibility

The standard deviation of single determination calculated on the first 17 postoperativ duplicate estimations in the present material was ± 4.7 ml corresponding to coefficient of variation of 2.0 . There was no significan difference between values obtained by the Krogh and Douglas methods.

Spirometry

Apparatus

Functional residual capacity was measured by the closed circuit helium dilution method (Meneely & Kaltreider 1941 1949 Holmgren, 1954). The thermal conductivity of the gas in the system measured with catharometer[1]) indicated the concentration of helium. The investigations were made with an 8-liter cylindrical spirometer[2]) with dead space of the system of 2.3 liters. On the expiratory side before the bell the apparatus is equipped with blower set to rate of 40 l min. In shunt on the expiratory side membrane pump forces portion of the gas at speed of 0.75 l min through water bottle to the catharometer and back to the main tubing. The capacity of the carbon dioxide absorber suffices for 10 to 15 examinations. The pressure fluctuations in the system are of the order only of some mm mercury even at large changes in the setting of the blower. Some of the earliest preoperative investigations were made on the same principle but with another apparatus[3]).

Procedure

After rinsing of he spirometer system with air from tube, oxygen, helium and air were added in the proportions of 1-4:5 to total volume of about 2 liters. The patient was then connected to the system and oxygen was continuously added at rate matching the oxygen consumption of the patient. The helium concentration was read on the catharometer every minute and, when the values had remained mainly unchanged for 3 minutes, the mixing of helium in the system was regarded as complete and the patient was asked to make two maximal expirations at about minute's interval. These recorded expiratory reserve volumes were used in the calculations of the residual capacity. The change from initial temperature in the system was noted. After period of rest and filling of he bell with air the patient was instructed to breathe calmly in the spirometer for about one minute. A short while after tw further maximal expirations, he was requested to make maximal inspiration immediately followed by maximal expiration, and to repeat the same procedure hree times at intervals of about 30 seconds. The mean value of the two largest amplitudes was usually used in the calculations. Some patients, however got tired and could only make one deep inspiration followed by one deep expiration, so that in these cases only one amplitude was measured.

Comments

Errors

Errors of this spirometric method were discussed by Meneely & Kaltreider (1941), Holmgren (1954) and Meneel et al. (1960). No corrections for absorption of helium were made in the present study. Birath & Swensson (1956) found that in spirometric investigations lasting seven minutes correction for the absorption of helium changed the calculated lung olume by 105 ml$_{BTPS}$.

The reproducibility of this method was studied at his laboratory by Holmgren (1954) with duplicate determinations of functional residual capacity in 11 normal individuals and 10 patients with emphysema. The standard deviation of single determination was ± 89 ml.

Technique of heart catheterization

Procedure

Catheters

Before heart catheterization the patients had light breakfast and premedication of 0.30 g quinidine, 0.10 g pentobarbital and penicillin. The investigation was started in the morning. On the previous day the patient had performed an exercise test in supine position on the catheterization table. He was thus familiar with the room and with the staff. Several patients

[1]) Cambridge Instrument Co.

[2]) Kifa, Stockholm.

[3]) Godart pulmotest.

had one or more pillows under the head during catheterization, while a few needed a pillow under the shoulders at the preoperative in vestigation. In the investigations before the heart operation a polyethylene catheter of length about 35 cm inside diameter 1 14 mm and outside d ameter 1.57 mm, was inserted in the brachial artery percutaneously (Sel dinger 1953) All invest gations after the heart operation were made with a telfon catheter of length about 140 cm inserted in the same way and with inside diameter 1.0 mm and outside diameter 1.8 mm. This long catheter was usually introd ced into the femoral artery on a metal leader on the end of the catheter a loop had been made (Littman et al 1960) to avoid i troducing the catheter into a coronary artery

Through a brachial vein a heart catheter was passed by the usual technique into the pulmonary artery Almost all examinations after the heart operation were made with a single-l men catheter no. 7[1]) (internal volume 1.5 cc) and most of the preoperative examinations with a double-lumen catheter no 9) (interval va lume 0 9 cc) In all the preoperative investiga tions the oxygen saturation was determined in samples from the ena cava sup. and compared with the o ygen saturation in the pulmonary artery for disclosure of significant left to-right shunts. The determinations of cardiac output were made on the F'ck principle after recording of the pressure n the right atrium, right entricle, pulmonary artery and a systemic artery

Cardi utput at rest

Exp ratory gases were sampled during 8 m nutes i a Dougla bag The respiratory val e was of the same type as used in deter m nat on of the basal metabol rate by the Do glas method. Checks were always mad fo leakage past the mouthp ec and through the nose on account of ll f tting noseclip. After f ve min tes of sampling in the bag blood samples were drawn sim ltaneously from the ma n stem of the pulm nary rtery and from the systemic artery The time for blood sampling was 30 to 60 sec and th amount of blood 7—8 cc in each syringe. The heart rate was recorded electrocardiographically during blood sampling.

Pressure recording

The pressure in the systemic artery was recorded from the brachial artery in most of the preoperative in estigations and from the ascend ng aorta in most of the postoperative. In almost all the latter cases the long teflon catheter was introduced into the left ventricle after the termination of gas sampling at rest. Pressures in the left ventricle were recorded with several amplifications, and in some cases a new determination of cardiac output was made with sampling from the left ventricle. In some of the preoperati e investigations the pressures in the left atrium and in few cases also in the left ventricle were recorded after ntroduction of a thin catheter by th trans bronchial technique, but such pressures are not directly comparable with those recorded in the other ways on account of the often rather great changes in the "basal state (Kar löf & Jonsson, 1963) and also on account of d fferent characteristics of the catheter-manometer systems. In these cases only pressures recorded at rest before the introduction of the bronchoscope, and during exercise only pressures transmitted through the catheters defined above, were used.

After investigations t rest a bicycle ergometer was fixed to the table and pressures were recorded with the patient s feet on the pedals.

Th performa ce of the exercise test in supine position has been described in an earlier chapter The load or loads were chosen after evaluating the exercise test in supine position wh ch th patient had performed on the preceding day The catheter the pulmonary rtery was usually placed in wedge position immediately before the patient started to work and then the wedge pressu e was continuously recorded during the first three minu es of exercise. When teflon catheter had been introduced int the left ventricle the tip of the catheter was left in the ventricle d ring the first 3—4 minutes of work If the catheter did not cause series of extrasystoles. The pressures in wedge position and the left ven

[1]) USC ray U.S.A.

trade were recorded simultaneously with the same amplifications and with the same zero line. Af er withd wal of the catheter from wedge position and from the left ventricle, respectively the cardiac output determination was performed in the same w y as at rest but with gas sampling period of 3 minutes and in some cases of marked dyspnea only 3—5 minutes. Low resistance valves were used during these sampling periods. In many of the preoperative examinations the patient worked at several loads.

And ss f gas sample

The echniques of gas analysis and of measurements of the gas olumes in the Douglas bags are described in an earlier chapter The Douglas bags were emptied within 20 minutes after sampling.

Analys f blood sample

The degree of oxygen saturation of hemoglobin in blood samples was determined by the spectrophotometric technique (Drabkin & Schmidt, 1945, Drabkin et al 1949 modified by Nahas et al 1950, Nahas 1951) in hemolysed blood with the extinctions read wavelengths 503 and 475 mμ. The technique with modifications was described by Holmgren & Pernow (1959). The concentration of hemoglobin was determined as described above. The oxygen capacity of the hemoglobin was calculated from the concentration curv of oxyhemoglobin and the degree oxygen binding capacity of hemoglobin (1.34 ml g) The amount of physically dissol ed oxygen in plasma was estimated from the dissociation curv of ox hemoglobin and the degree of oxygen saturation After blood sampling the syringes were immediately sealed with mercury inserted in icewater and then placed in refrigerator The blood was analysed within some hours of sampling The dead space of each syringe was corrected for Before the sampling the dead space was filled with heparin.

Equipm nt for pre ss re recording

In all preoperative catheterizations resistance wire strain gauge electromanometers[1]), nd in almost all postoperative investigations variable inductance manometers[2]), were used for the pressure measurements together with al amplifiers[3]). The pressures were always recorded with 4-channel electrocardiograph with optical galvanometers. Electrocardiograms and 2 or 3 pressures wer recorded simulta neously and were continuously follow ed on an oscilloscope. The positions of the catheters were observed on he image intensifier or on television screen. All pressures were referred to level 5 cm dorsally to the sternum in the fourth intercostal space

Calib atio

I most of the preoperative investigations hydrostatic pressures from elevated water bot tles ere recorded interchangeably with the blood pressures during catheterization. In most of the postoperati examinations use was made of electric standards which were frequent ly calibrated against hydrostatic pressures and checked for linearity The greatest deviation between two successive calibration was ± 2 at full scale deflection at water pressure of 100 mm mercury Dynamic calibration of the catheter-manometer system was performed by recording pressure waves generated by pump with large frequency range. By withdrawing the plunger of specially constructed syringe the response to transient pressure waves was studied. The amplitude frequency curves of the heart catheter-manometer system used for right heart catheterization showed an equal response up to 2—10 c/ with the degrees of damping used during the catheterizations and with two kinds of manometers tested in he system[1] [2]). The corresponding range for the teflon catheter manometer ystems used for left heart catheterization was 5—10 /s. The undamped natural frequencies for the catheter manometer systems were 10—20 and the degree of damping 0.1—0.7

With the highest sensitivity of the manometer amplifier-recorder system pressure of 10 mm mercury was recorded with linear response all over the paper width of 10 cm.

[1]) EMT 456, Elema-Schönander AB.
[2]) EMT 490 A, Elema-Schonander AB.
[3]) EMT 460, Elema-Schönander AB.

The three paper speeds used for pressure recording were 4 40 and 100 mm/s. To check the stability of the zero line, this was recorded both before and after each recording of pres sures.

During exercise the *first pressure recording* at each load was made between the third and fifth minutes, and the second after the deter mination of cardiac output usually 8—10 minutes after beginning work at the load. The pulmonary wedge pressures were usually recorded immediately before the determination of cardiac output. The mean pressures were recorded by means of electrical integrators with a time constant of 0.8 sec.

Calculation of pressures

All pressures were averaged over several respiratory cycles and usually measured on *curves recorded at the lowest paper speed*, and an analysis of the configuration of the curves was always made on adjacent parts recorded at the h gher paper speed From curves with wedge pressure and left ventricle pressure recorded simultaneously and with common zero l ne and the same high amplif cation the area between the wedge and left ventricle pressure *curve was copied on translucent paper with* correction for the time delay of one curve in relation to the other One of the curves was thus moved parallel to the time a is until the f rst rapid descent of the v wave coincided with the descending part of the left ventricle curve After this correction the area was measured on the transl cent paper with a planimeter The corrections corresponded to time d fferences arying from 0 t 0.04 sec. The mean pressure gradient during d astole over the mitral ostium was calculated by dividing the area by ts length al ng the time axis and multiplying by the amplification f ctor Mean values of such calculat ons from 10 heart cycles were used in most cases.

The time dur tion of the diastolic pressure gradients in those heart cycles was alculated and was assumed to coincide with the diastolic f lling period d ring this time An index of mitral area was calculated as a quotient between mean value of olume of flow during diastole () in ml/sec and the square root of the mean pressure gradient (p) in mm Hg $\frac{}{\sqrt{p}}$

Comments

Cathete m nom tersystem

Frequency response

In only two catheterizations in the present investigations was the heart rate during exer cise higher than 150 beats/min on recorded curves used for pre- and postoperative compa risons. If pressure waves are adequately described by Fourier analysis up to the sixth harmonic (Hansen, 1949) an undamped natural frequency of the catheter manometer system of 15 c/s would suffice for a satisfactory record at that heart rate, and for recording at rest half of that natural frequency would be suff cient. The undamped natural frequency of the catheter-manometer system used in the present study was thus satisfactory for ana lysis of pressure curves recorded at rest, but often somewhat low for recording d ring exer cise. As no analysis was made of the configuration of pressure curves during work, the frequency response of the catheter manometer system does not seem to introduce any signifi cant error in the measurements.

D gree of damping

Most catheter-manometer systems are under damped oscillating systems. As the phase-shift is proportional to frequency only n critical damped systems (degree of damping of 0.7), there are different degrees of phase shift of the Fourier components in the pressure curves in most recordings. At low degrees of damping, however the phase h ft is small and according to McDonald (1960) the best way of recording blood pressures with great accuracy for both ampl tude and phase response is a catheter manometer system with a high natural frequency and a very small degree of damping.

Wood et al. (1954) studied the effect of artificially generated motion rtefacts on different catheter manometer systems They found that catheter manometer systems with a uniform dynamic response up to 5—10 c/ were least sensibl to high frequency artefacts. Thus f r reduction of such artefacts th catheter manometer system used in this examination was suitable.

Analy is f pre sur curve

Only wedge pressures with characteristic shape and time relations to the electrocardio-

gram examined on the oscilloscope were recorded. Often the position of the catheter was changed several times before characteristic wedge pressure curve was observed on the oscilloscope, and in some patients with high resistance in the pulmonary vessels it was not possible to get such curve. Sometimes, in cases of atrial fibrillation, it may be difficult to differentiate between wedge pressure curve and a curve recorded from the peripheral pulmonary artery. Only curves with wave of maximal amplitude simultaneously with or somewhat later than the incisura of the pulmonary artery curve, or definitely later than the T wave of the electrocardiogram, were accepted as wedge pressure.

Time delay

If left atrial pressure is considered to be well reproduced by recording in wedge position, the accuracy in calculating diastolic pressure gradient over the mitral valve from wedge pressure curve and left ventricle curve is to some degree dependent on the time delay of the curves. The time delay of the pulmonary wedge pressure curve depends on the catheter and also on the anatomy and functional state of the pulmonary vessels. On account of the variable time delays in different patients the pressure curves were not related to electrocardiogram or phonocardiogram.

Estimation of cardiac output

The error in measurement of blood flow on the Fick principle by using blood samples which are time averages and not volume averages was discussed by Visscher & Johnson (1953). If in measuring blood flow through an organ the difference in concentration of the reference material in inflowing and outflowing blood is constant during the time of measurement, there is no error of that kind in spite of changing flows. According to Wood et al. (1955) no phasic variations in oxygen saturation in the mixed venous blood of significant degree occur in man during the cardiac cycle. The latter authors, however, assumed that small cyclical changes in oxygen saturation with the phase of respiration could introduce an error which might be more apparent during exercise. Donald et al. (1954) showed that the differences in arterial venous oxygen difference from minute to minute in material of rheumatic heart disease were very small both at rest and also after some minutes of exercise. They estimated the percentage error as between ± 3 and ± 4 % in flow determinations using the mean arteriovenous oxygen difference with respect to time in 3 patients with mitral stenosis. They concluded that the changes in flow and in A—V oxygen difference and in resulting sampling errors have been greatly overestimated. In spite of different kinds of errors the Fick method remains the most reliable at present (Harris & Heath, 1962).

Steady state

The requirement for measurements of blood flow according to Fick is relative steady state in oxygen consumption and in the circulatory adaptation during the measurement. In the study by Donald et al. (1954) the patients with rheumatic heart disease were divided into three groups according to the level of work they could sustain for 5 minutes. Nine of the twelve patients in the first two groups had reached steady state after three minutes in respect of cardiac output, A—V oxygen difference and oxygen uptake during exercise close to the tolerance level, one after 4 minutes' exercise, and two had not reached steady state after 5 minutes' exercise. In the present material the level of exercise related to the patients' maximal performance was lower than in that study in almost all cases.

Statistical methods

Statistical calculations were mainly performed according to Snedecor (1959). The difference between regression lines was in part tested according to Hald (1960). The following probability (P) levels of significance were used: $P < 0.001$*** highly significant, $0.001 \leq P < 0.01$** significant and $0.01 \leq P < 0.05$ probably significant. Generally the means are given ± standard error of the mean.

INVESTIGATIONS AND GENERAL PROCEDURE

During the investigations the patients were treated in the Medical the Thoracic Medical or the Thoracic Surgical Clinic of Karolinska sjukhuset. An extensive history was taken and a clinical examination was made, blood and urine were analysed according to the usual routine and antistreptolysin and antistaphylolysin titres in blood were determined in all patients At the Clinical Physiological Central Laboratory or the Clinical Physiological Laboratory of the Thoracic Clinic the following records were kept for practically all patients both pre and postoperatively electrocardiogram at rest during and after maximal or submaximal work on bicycle ergometer blood volume oxygen uptake at rest, phonocardiography and lung volumes by spirometry Right heart catheterization was done at rest and if the condition of the patient permitted also during work in most patients postoperatively and in all patients preoperatively according to the criteria for selection. Conventional chest radiograms were taken at the Department of Diagnostic Radiology both in erect position with different projections and in supine position

The preoperative examinations were made during the years 1957—60 During this time I worked for three years in the Clinical Physiological Central Laboratory and one year in the Clinical Physiological Laboratory of the Thoracic Clinic at Karolinska sjukhuset took part in a large number of the preoperative clinical physiological examinations and performed some of the catheterizations and recorded pressures during others. Fifty five of the postoperative examinations were performed during the period January 1962—January 1963 Fifty-two of these postoperative investigations, including 50 heart catheterizations were done by me The catheterizations were performed in cooperation with Dr Lars-Göran Ekelund. Fifty two of the patients were hospitalized for a week in the Medical Clinic of Karolinska sjukhuset, the other eight patients were treated for longer periods either in the Medical or in the Thoracic Clinic. Pre and postoperative examinations were performed by the same methods and according to the same standardized procedure At the postoperative examinations combined right and left heart catheterization was performed in 38 cases. The exercise tests made on 55 of the patients were supervised by me. They were made in sitting and in supine position on different days and at the beginning of the week in which the patient was hospitalized. Heart catheterization was performed at the end of the same week. If a patient was treated with digitalis quinidine and diuretics separately or in combination before admission to hospital, this treatment was unchanged during the period of investigation

PREOPERATIVE INVESTIGATIONS

Histories

Some data from the patients histories are summarized in table 7 Twenty-three patients knew that they had had a rheumatic infection, 21 of them had had rheumatic fever and 2 chorea. The average age at the time of the rheumatic infection was 14 years, the lowest age 4 years (case 12 had chorea at 4 years and rheumatic fever at 15 years of age)

The verage time between the rheumatic infection and the heart operation was 28 4 years with a range of 11—42 years.

The mean age at the time when the heart disease was diagnosed was in the whole group 28 4 years, range 4—55 years.

Three patients had no dysnea on effort before the heart operation, the other 57 patients had had dyspnea on effort during a mean period of 9.9 years before operation, range 0.5—45 years.

The average age of these 57 patients when this dyspnea started was 30.8 years range 6—55 years.

Twenty-one patients had atrial fibrillation before the heart operation with an average duration of the arythmia before operation of 6.3 years in 20 cases with a longest interval of 20 years. One patient (case 19) did not know when the irregular rhythm started, but probably it was more than one year before the operation.

Table 7
Anamnestic data

			–	Range
Rheumatic fever or chorea	Number of years befor op	23	28.4	11—42
	At age	23	14 0	4—28
Rheumatic fever or chorea unknown		47	—	
Diagnosis of valvular disease for the first time	Number of years before op	60	11,6	0.3—39 0
	At age	60	28.4	4—55
Incipient dyspnea on exertion	Number of years before op.	57	9 9	0 5—45.0
	At age	57	30 8	6—55
No dyspnea on exertion		3		
Beginning of atrial fibrillation of long duration	Number of years before op.	20	6.3	0.3—20.0
	At age	20	38 2	27—52

The mean age of the 20 cases at the start of the arrythmia was 38.2 years, lowest age 27 years.

Sixteen of the patients had had hemophthysis, 4 pulmonary edema and 8 a history of systemic emboli. Ten patients were cholecystectomized, three others had gallstones diagnosed by X-ray.

Other diseases of importance in the present material are seen in table B in appendix.

Comparisons with other materials

The incidence of known active rheumatic state in cases with relatively pure mitral stenosis studied by Wood (1954) was 60 %. In the autopsy series of mitral stenosis reported by Hall (1961) the incidence of *firm rheumatic fever* was 33.7 % in females and 35.0 % in males. The latter figures agree well with the incidence in the present material (acute rheumatic fever in 35.0 %).

The mean age at the initial rheumatic attack in Wood's patients with mitral stenosis was 12 years, in the present material 14 years and in Paul Hall's material 15.6 years.

The incidence of atrial fibrillation at the time of heart operation in Wood's material was 41 % and in the patients studied by Otto (1964) 41.9 %, in the present material the figure was 35.0 %.

In a material of surgical cases (Wood, 1954) 14 % of the patients had a history of systemic emboli, which is about the same as in the present material (13.3 %). In 1000 operated patients with mitral stenosis Ellis et al. (1959) found 18.6 % who had had attacks of peripheral embolisation prior to surgery.

A higher incidence of gallstones in patients operated for mitral stenosis was observed by Glenn & Redo (1958). They reported the incidence to be 14.3 % in 300 operated cases. The corresponding figure in the present material was 21.7 %.

Clinical findings

Manifest peripheral edema was found in only one patient (case 55). No patient was confined to bed.

The degree of disability in the patient's everyday life was estimated from the history and the patients were grouped according to the classification of N.Y.H.A. No patient had dyspnea of moderate or marked degree at rest. The numbers of patients in the four groups were: I 6, II 19, III 29, IV 6.

Forty nine of the patients had been treated with digitalis, most of them for many years. The reasons for the medication seemed in most cases to have been the existence of valvular disease in combination with dyspnea on exertion. Many of the patients had also been treated with quinidine and diuretics.

Phonocardiographic findings

Sounds

The incidence of heart sounds and heart murmurs is shown in table 8. Thirty-nine patients had sinus rhythm and 21 atrial fibrillation. Three patients (cases 51, 54, 57) had a clearly visible third sound. This sound is here defined as localised vibrations which decrease to an intensity of 1/3 within 0.10 sec in the lowest frequency band (25 c/s) and have the temporal relationship of a third heart sound.

Three patients (cases 15, 23, 38) had a pulmonary ejection sound which was considered to exist only when it had its maximal intensity over the pulmonary area and when the sound could certainly be separated from the components of a delayed first sound.

Table 8
Preoperative phonocardiographic findings at rest in 60 patients

		Number
Rhythm	Sinus rhythm	39
	Atrial fibrillation	21
Sounds	Marked third sound	3
	Pulmonary ejection sound	3
	Distinct opening snap	50
	No distinct opening snap	10
Murmurs	Pansystolic high-frequency with maximal intensity over apex	7
	Pansystolic high-frequency with maximal intensity over lower end of sternum	2
	Diastolic apical murmur	54
	No distinct diastolic apical murmur	6
	Ejection murmur of stenosis type with maximal intensity over I 2 dx	5
	Early diastolic high-frequency regurgitation murmur with maximal intensity over heart base	9

Table 9
Type of apical diastolic murmur on phonocardiogram in 54 patients

	Number
Distinct onset immediately after opening snap	32
Starting point at the time of the third sound	15
No distinct starting point at opening snap or at the third sound	5
Murmur only visible in presystole	2
Total	54
Increasing intensity and frequency in presystole in the 39 patients with sinus rhythm	23

Fifty patients had a clearly visible opening snap 10 had not in one of the latter an early diastolic regurgitation murmur of high intensity may have concealed an opening snap and in another the possibility of a paradoxal splitting of the second sound and very irregular rhythm with varying time relations and intensities of the sounds made the diagnosis of an opening snap difficult.

Murmurs

The incidence of different murmurs was as follows. A diastolic murmur not beginning immediately after the second sound with the maximum over the apex was recorded in 34 patients, no such murmur in six patients, three of whom had an opening snap and three had not (cases 18, 32, 44).

The starting point of the apical diastolic murmur is seen in table 9 In 54 patients with an apical diastolic murmur, this began immediately after the opening snap in 32 cases and at the time of a third sound in 15 cases 2 patients had only an atriosystolic (presystolic) murmur. In the remaining 5 cases the starting point of the murmur was difficult to define.

In 23 of the 39 patients with *sinus rhythm* there was marked *atriosystolic* murmur. This is defined here as a murmur which during the time of mechanical atrial systole increases in intensity threefold. To avoid confusion with the first component of the *first* sound, the measurements of intensity were made up to the Q or R wave. The evaluation of atriosystolic murmurs was made solely on curves recorded in supine position at rest.

Pansystolic high-frequency murmur of *regurgitation type with maximal intensity* over the apex and indicating mitral insufficiency occurred in seven patients in one of them the murmur started and ended with a sound (case 18) and was thus not completely pansystolic. The same kind of murmur but with maximal intensity over the lower part of the sternum and indicating tricuspid insufficiency was recorded in two patients (cases 36 55)

A systolic high-frequency murmur of stenosis type over the aortic region was found in 5 patients and an early diastolic regurgitation murmur over the heart base was recorded in 9 patients

Time relations

Some time relationships are shown in table 10 The time between the beginning of the Q wave and the first vibrations of medium to high frequency and of high amplitude (Q—1 *interval*) was in 59 patients

Table 10
Time relations on preoperative phonocardiograms

	n	$\bar{x}$	σ	range
Q — 1 interval in seconds	59	0.081	0 002	0.04—0.11
2 — OS interval in seconds	50	0.073	0 003	0.04—0 11
Proceeding R — R interval in seconds	59	0.82	0.02	0.50—1.50

0 081 ± 0 002 sec with R—R interval of preceding heart cycle of 0.82 ± 0 02 sec.

There was no significant mean difference in Q—1 interval between the groups of patients with sinus rhythm and those with atrial fibrillation.

The time between the first vibrations of medium to high frequency in the first component of the second sound and in the opening snap (2—OS interval) was in 50 patients 0 073 ± 0 003 sec with the same R—R interval of preceding heart cycles as above.

Comments

Absence of opening snap

The incidence of cases without clearly visible opening snap was 8 of 58 if two cases with uncertain findings are eliminated. This figure is somewhat higher than in some *other materials*, e.g 6 of 106 consecutive patients undergoing mitral valvulotomy (Wells 1957) and 1 of 33 patients (Mounsey 1953) To some extent this discrepancy may be explained by different definitions of the recorded opening snap In three of the eight patients in the present material there were some uncharacteristic vibrations of low amplitude which might have been caused by opening of the mitral valve, in the other five patients there were no such vibrations.

Absence of apical diastolic murmur

The incidence of mitral stenosis without diastolic murmur varies greatly in different materials. This was to be expected since mitral stenosis may not be diagnosed if this murmur is absent. True mute mitral stenosis is regarded as very rare by several authors (McKusick, 1958) Levine & Love

(1952) collected 19 cases of mitral stenosis without diastolic murmur and they assumed that such cases might well be between 5 to 10 % of all cases of mitral stenosis. In these cases, however phonocardiograms were not recorded. Kelly (1955) observed no diastolic murmur in 17 of 76 patients with mitral stenosis.

Atriosystolic murmur

Variations in the first part of the first sound may sometimes be confused with an atrial systolic murmur. Such variations always start after the Q or R wave (Allmurung, 1949).

In many cases without a diastolic murmur recorded at rest, such a murmur may be recorded after exercise (Pinardi & Ström, 1959). The filling of the left ventricle occurs during the whole of diastole in cases with tight mitral stenosis. When the heart rate is increased, the duration of diastole is shortened relatively more than the duration of systole. The volume of blood which passes the mitral ostium per time unit is then increased even when the cardiac output is unchanged due to decreasing stroke volume. An increased flow gives better conditions for murmur. In some of the cases without diastolic murmur at rest in the present material such murmur was recorded after exercise but as phonocardiographic recording after exercise was not performed in all cases before operation these investigations were not analysed in the present study.

Time relation

As both Q—1 and 2—OS intervals are related to the length of the preceding heart cycle, these intervals should be corrected for varying duration of previous cycles before inter or intra-individual comparisons are made. This was done by Wells (1957) who used corrected Q—1 and 2—OS intervals. Such corrections require values of Q—1 and 2—OS intervals after heart cycles of varying duration. It was not possible to obtain such variations in cycle duration in all patients both before and after operation in the present material. As all Q—1 and 2—OS intervals in each patient were measured after heart cycles of equal duration, the error caused by varying duration of preceding heart cycles pre and postoperatively was eliminated in intra individual comparisons. The Q—1 intervals were consistent with those reported by Weissler et al. (1958) in a material of ten cases of mitral stenosis with a mean value of 0.080 ± 0.003 sec and R—R mean interval of 0.80 ± 0.03 sec. The same authors found the mean value of Q—1 interval in 18 healthy persons to be 0.055 ± 0.002 sec at R—R intervals of 0.81 ± 0.04 sec. In a material of 75 patients with mitral stenosis (Kelly 1955) the mean value of the Q—1 interval was 0.09 ± 0.003 sec.

The Q—1 interval was shown to be increased in hypertensive patients by Bayer et al. (1956) and Weissler et al. (1958). In the present material there were 3 patients with mean pressure above 110 mm Hg at the preoperative catheterization (cases 19 25 57). The Q—1 intervals of these patients were not different from the rest.

The 2—OS intervals in the present material agree well with those of 33 patients with mitral stenosis studied by Mounsey (1953) who found mean value of 0.07 sec, range 0.03—0.14 sec: cycle length was not reported. In a material of ten patients with mitral stenosis Weissler et al. (1958) found mean value of 0.08 ± 0.001 sec at an R—R interval of 0.8 ± 0.03 sec.

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Comments

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Absence of apical diastolic murmur

The incidence of mitral stenosis without diastolic murmur varies greatly in different materials. This was to be expected since mitral stenosis may not be diagnosed if this murmur is absent. 'True mute mitral stenosis is regarded as very rare by several authors (McKusick, 1958) Levine & Love

Table 13
Heart volume in ml in supine position
Preoperative studies

Sex	Rhythm	n	$\bar{x}$	ε	$\bar{d}$	P
♂	Sinus	12	1096	38	930	$p<0.001$
	Fibrill.	5	2026	348		
	Sinus + fibrill.	17	1369	144		
♀	Sinus	24	873	31	261	$p<0.001$
	Fibrill.	15	1134	60		
	Sinus + fibrill.	39	974	58		
♂ + ♀	Sinus + fibrill.	56	1094	55		

stenosis patients for comparison in which maximal work intensity has been measured in this way and the same applies to the measurement of $\frac{W_6}{\Delta f}$

The numbers of patients with congestive heart failure and of those treated with digitalis are dealt with in the section on clinical findings.

Heart volume

The mean value of heart volume in supine position in 12 men with sinus rhythm was 1096 ± 38 ml and in 5 men with atrial fibrillation 2026 ± 348 ml, in 24 women with sinus rhythm 873 ± 31 ml and in 15 women with atrial fibrillation 1134 ± 60 ml (table 13). These mean values were all outside normal variation. The differences in heart volumes between the groups of patients with sinus rhythm and with atrial fibrillation were highly significant.

Comments

The values of heart volume are very similar to those of 21 patients with mitral stenosis selected for mitral valvulotomy studied by Linderholm & Strandell (1958). Sex ratio and incidence of sinus rhythm and of atrial fibrillation are about the same in both materials.

Comparisons with other materials in which radiological technique and methods of calculation have differed, are difficult to evaluate.

Total amount of hemoglobin, blood volume and concentration of hemoglobin

The mean value of total hemoglobin in g/kg body weight in 39 women was 9.5 ± 0.3 in 16 men 10.2 ± 0.4, and in both women and men 9.7 ± 0.2 (table 14).

The lowest value of total hemoglobin was 6.9 g/kg (case 57) and 6 women had values lower than 8 g/kg. The highest value was 13.9 g/kg (case 62)

Table 12

Maximal work intensity $\frac{W_6}{\Delta f}$ *Preoperative values*

	Sex	Rhythm	n	$\bar{x}$	ε	$d_{\bar{x}_s - \bar{x}_f}$	P
Sitting kpm/min.	♂	s	13	545	54	+166	0.2—0.1
		f	4	379	108		
		s + f	17	504	50		
	♀	s	26	357	26	+64	0.2—0.1
		f	15	293	28		
		s + f	41	333	19		
Supine kpm/min.	♂	s	12	354	58	+112	0.5—0.4
		f	3	242	156		
		s + f	15	332	50		
	♀	s	25	208	25	+23	0.6—0.5
		f	14	185	25		
		s + f	39	195	18		
Sitting Kpm/min/pulse beat	♂	s	11	6.1	0.6		
	♀	s	24	5.3	0.4		
Supine Kpm/min/pulse beat	♂	s	9	6.6	0.7		
	♀	s	21	4.2	0.4		

s = sinus rhythm f = atrial fibrillation

Work intensity — pulse difference ratio, $\frac{W_6}{\Delta f}$

The ratio $\frac{W_6}{\Delta f}$ kpm/min/pulse beat was in sitting position in 11 men 6.1 ± 0.6 and in 24 women 5.3 ± 0.4. The corresponding values in supine position were in 9 men 6.6 ± 0.7 and in 21 women 4.2 ± 0.4. There was no mean difference of any significance between $\frac{W_6}{\Delta f}$ in sitting and in supine position in the groups of males and females.

Comments

The values of maximal work intensity are definitely low compared with normal values. The mean final work load (1004 kpm/min) in sitting position in 30—49 years old males (Strandell, 1964) was thus twice as high as in the present material although the work intensities were not maximal. In 40—49 years old females the maximal oxygen uptake during exercise in sitting position was 2.01 ± 0.07 l/min (Åstrand, 1960) corresponding to about 700 kpm/min.

I have found no other materials of mitral

Comments

The results agree well with those of Resnik & Friedman (1935) in their group III, consisting of heart patients without signs of cardiac failure.

Wilson et al. (1954) observed in 9 heart patients without manifest edema an excess of sodium and of water with a mean increase in body weight of about 4 % compared to healthy individuals. If, for instance, sodium and water retention increases body weight by 5 kg, the predicted value of basal metabolic rate according to the tables of Harris & Benedict will be over-estimated by about 4—6 %.

Spirometry

Predicted values were calculated from age, body length and body weight from regression equations according to Grimby & Söderholm (1963). The predicted values were converted from ATPS to BTPS conditions by using a factor of 1.10. Individual differences between predicted and observed values were calculated.

Compared with predicted values there were in 57 patients highly significantly lower values of vital capacity, functional residual capacity and total capacity with mean differences of 954 ± 81, 432 ± 85 and 959 ± 101 ml respectively (table 15). The residual volume was not significantly changed. The ratio of residual volume to total capacity was highly significantly increased, with a mean difference of 5.2 ± 0.8 %. The increase in the ratio of functional residual capacity to total capacity was significant with a mean difference of 2.5 ± 1.0 %.

Comments

Studies of total lung capacity with its subdivisions in patients with mitral stenosis

Table 15

Preoperati prometric da s

Differenc s between b erved and predicted values

Lung volume		$\bar{x}$	$s_{\bar{x}}$	P
Vital capacity ml	57	−954	81	<0.001
Residual volume (RV) ml	57	±0	50	>0.9
Functional residual capacity ml (FRC)	57	−432	85	<0.001
Total capacity (TC) ml	57	−959	101	<0.001
$\frac{RV}{TC}$	57	+5.2	0.8	<0.001
$\frac{FRC}{TC}$	57	+2.5	1.0	0.01—0.02

Table 14
Total amount of hemoglobin (THb) and blood volume (BV) in relation to body weight (Bwt) and concentration of hemoglobin (Hb conc.)
Preoperative values

	Sex	n	$\tilde{x}$	ε	$\bar{d}$	P
THb / Bwt g/kg	♂	16	10.2	0.4	0.7	>0.3
	♀	39	9.5	0.3		
	♂ + ♀	55	9.7	0.2		
BV / Bwt ml/kg	♂	14	82.9	2.4	1.1	>0.5
	♀	39	84.0	2.5		
	♂ + ♀	53	83.7	1.9		
Hb conc. g/100 ml blood	♂	14	13.1	0.3		
	♀	39	12.2	0.3		

The mean value of the blood volume in ml/kg body weight in 39 women was 84.0 ± 2.5, in 14 men 82.9 ± 2.4, and in both women and men 83.7 ± 1.9.

Neither in respect of total hemoglobin nor of blood volume were the differences in mean values between males and females significant. The mean value of concentration of hemoglobin in 39 women was 12.2 ± 0.3 g % and in 14 men 13.1 ± 0.3 g %.

Comments

The mean value of total hemoglobin in men did not deviate significantly from that of 26 healthy males, 10.4 ± 0.2 g/kg studied at this laboratory (Ekelund 1962). The corresponding mean value in women was 93 % of that in men in the present material, which is a normal relation.

The mean value of blood volume calculated from total hemoglobin in the 26 healthy males studied by Ekelund was 81.9 ± 1.7 ml/kg bwt. There was thus no significant difference from the corresponding value in the present material.

The mean values of concentration of hemoglobin did not differ significantly from normal values at this laboratory (13.6 ± 0.8 and 13.2 ± 0.8 g/100 ml in 30—39 and 40—49 years old men respectively (Strandell 1964).

Basal metabolic rate

In 54 patients the mean quotient between the values of basal metabolic rate measured by the closed Krogh method and those predicted from the tables of Harris & Benedict (1919) was 1.02 ± 0.01.

Only two patients (cases 8 and 53) fell outside the range of ± 15 % from predicted value, a range often considered as the normal variation.

Table 16

Data from preoperative catheterization at rest

		n	$\bar{x}$	$e_{\bar{x}}$	$\bar{d}$	$e_{\bar{d}}$	P
Arterial oxygen saturation in per cent	All patients	60	96.4	0.5	—	—	—
A—V oxygen difference	All patients	60	48.9	1.8	—	—	—
	Patients with sinus rhythm	40	45.0	1.9			
ml/l	Patients with atrial fibrillation	20	56.7	3.0	11.7	3.4	0.01—0.05
Cardiac index l/min/m²	All patients	60	3.24	0.06			
	Patients with sinus rhythm	40	3.53	0.13			
	Patients with atrial fibrillation	20	2.66	0.14	0.87	0.21	<0.001
Stroke volume ml	Patients with sinus rhythm ♂	13	84.1	5.5	—	—	—
	♀	27	63.4	3.8	—	—	—
Stroke volume/blood volume ml/l	Patients with sinus rhythm	37	13.9	0.6	—	—	—
Stroke index ml/m²	Patients with sinus rhythm	40	41.5	1.8	—	—	—
Mean pressure in right atrium mm Hg	All recordings	45	3.7	0.6	—	—	—
					1	0.77	0.2
Right ventricle end-diastolic pressure mm Hg	All recordings	59	4.7	0.5			
	Patients with sinus rhythm	39	4.6	0.5			
	Patients with atrial fibrillation	20	4.8	1.1	0.2	1.1	0.8—0.9
Pulmonary artery mean pressure mm Hg	All patients	60	34.3	1.8			
	Patients with sinus rhythm	40	34.6	2.4			
	Patients with atrial fibrillation	20	33.8	2.5	0.8	3.9	0.8—0.9

(Frank et al., 1953 Krautwald et al 1961) have shown that if the materials are grouped according to functional disability there was a gradual decrease of total capacity and vital capacity and a gradual increase of residual volume with increasing symptoms. In the present material there was no significant change in residual volume but the changes in the other lung volumes agreed well with corresponding changes in functional groups consisting of patients with symptoms of moderate degree in those studies According to Comroe et al. (1962) a normal residual volume is a characteristic finding in patients with mitral stenosis As the studies above were performed by other methods and the predicted values were calculated according to other formulae than in the present material, no further comparisons will be made

The spirometric values of total capacity and vital capacity change with body position The values are lower in recumbent position. Many spirometric examinations are made with the patient in the latter position. In the present material the patient was seated during the investigation.

If body weight is one of the variables in prediction of normal values, an error is introduced in patients with manifest or latent edema. In the regression equations for predicted values body weight is not a variable in prediction of residual volume vital capacity or quotient between residual volume and total capacity in women, nor for calculating vital capacity in men In the other equations where body weight is a variable an increase in body weight of 1 kg decreases the predicted value of functional residual capacity in males by about 41 ml and the value of quotients by up to 0 14 % In the rest of the equations the influence of body weight is less Among the investigated patients none had marked edema. The effect of manifest or latent edema will be too small predicted values, as body weight enters into the regression equations as a negative term. As the vital capacity and total capacity were highly significantly decreased in the present material, the degrees of significance of these changes were not changed by an eventual error of this kind.

Right heart catheterization

Preoperative values at rest (Tables 16, 17)

Oxygen uptake

Oxygen uptake during catheterization was compared with basal metabolic rate (BMR) usually measured some days before the catheterization in 57 patients. In 25 of the patients the Douglas bag values of BMR were used in this comparison, in the rest of the patients the Krogh values. The catheterization value was higher than the BMR value in 55 patients. The highly significant mean difference in per cent of BMR value was 20 9 ± 1.8 %

Arterial oxygen saturation

The mean value of the arterial oxygen saturation in all 60 patients was 96.4 ± 0 5 %

Arterio-venous oxygen difference

The mean value of arterio-venous oxygen difference in all 60 patients was 48 9 ± 1.8 ml/l. The A—V oxygen difference at rest and during work plotted as function of oxygen uptake in the whole group and the curvilinear regression with standard deviations from a normal material studied at this laboratory (Holmgren et al. 1960, Bevegård et al 1960) are seen in fig 3 In the 40 patients with sinus rhythm the mean

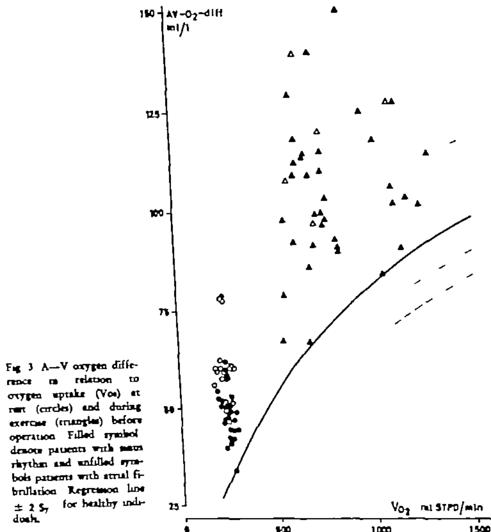


Fig 3 A—V oxygen difference in relation to oxygen uptake (V_{O_2}) at rest (circles) and during exercise (triangles) before operation Filled symbol denote patients with sinus rhythm and unfilled symbols patients with atrial fibrillation Regression line $\pm 2\,S_y$ for healthy individuals.

Table 17

Oxygen uptake ml$_{STPD}$/min (x) and cardiac output l/min (y) at rest Correlation and regression

		$\bar{x}$	$\bar{y}$		P	regr equation	S y.x
Sinus rhythm	40	250	5.87	0.528	< 0.001	y = 0 0192x + 1.07	1.74
Atrial fibrillation	20	238	4 39	0.260	> 0.1	— —	—
Normal material	27	276	7.61	0.559	0.001—0 010	y = 0 0177 + 2.73	1.09

Table 16
Table continued

		n	$\bar{x}$	$\varepsilon_{\bar{x}}$	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
Pulmonary wedge mean pressure mm Hg	All patients	59	21.8	0.9			
	Patients with sinus rhythm	39	22.5	1.0	2.1	1.8	0.2—0.3
	Patients with atrial fibrillation	20	20.4	1.7			
Pulmonary vascular resistance dynes sec cm^{-5}	All patients	59	220.8	30.4			
	Patients with sinus rhythm	39	196.0	36.8	74.4	64.5	0.2—0.3
	Patients with atrial fibrillation	20	270.4	55.2			
Systemic vascular resistance dynes sec cm^{-5}	All patients	60	1312.0	56			
	Patients with sinus rhythm	40	1143.2	54	500	91.2	<0.001
	Patients with atrial fibrillation	20	1643.2	70			
Right ventricle work against pressure kpm/min	All patients	60	2.23	0.13			
	Patients with sinus rhythm	40	2.44	0.17	0.62	0.26	0.01—0.05
	Patients with atrial fibrillation	20	1.82	0.13			
Brachial artery mean pressure mm Hg	All patients	60	84.2	1.7			
	Patients with sinus rhythm	40	79.5	1.4	15	3.1	<0.001
	Patients with atrial fibrillation	20	93.7	1.1			
Specific ventilation ml_{BTPS}/ml_{STPD}	All patients	60	33	1			
	Patients with sinus rhythm	40	34	1	2	2	0.4—0.5
	Patients with atrial fibrillation	20	32	1			

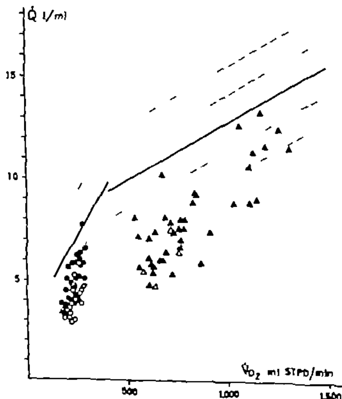


Fig. 4 Cardiac output ($\dot{Q}$) in relation to oxygen uptake ($\dot{V}_{O_2}$) at rest (circles) and during exercise (triangles) before operation. Filled symbols denote patients with sinus rhythm and unfilled symbols patients with atrial fibrillation. Regression line ± S for healthy individuals at rest y = 0.018 + 2.7 during exercise y = 0.006 + 7.0.

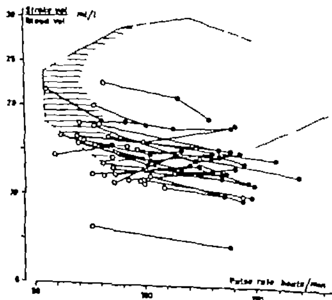


Fig. 5 Quotient between stroke volume and blood volume in relation to pulse frequency at rest (unfilled circles) and during exercise (filled circles) in patients with sinus rhythm before operation. Dotted line indicates normal range. Hatched area normal range at rest.

value was 45 0 ± 1 9 ml/l and in the patients with atrial fibrillation 56 7 ± 3 0 ml/l The difference between these values was significant.

Cardiac output

In the patients with sinus rhythm there was a significant correlation between cardiac output and oxygen uptake (r = 0 528) In the patients with atrial fibrillation there was no significant correlation The mean values of cardiac output and oxygen uptake were in the first group 5.87 l/min and 250 ml/min and in the latter group 4 39 l/min and 238 ml/min. In the group of patients with sinus rhythm the equation for cardiac output, l/min (Y) as a linear function of oxygen uptake ml/min (x) was Y = 0 019 x + 1 07 The relation between cardiac output and oxygen uptake in all patients and the regression line ± 2 Sy.x for 27 healthy individuals studied at rest and during exercise at this laboratory (Holmgren et al. 1960 Bevegård et al 1960) is seen in fig 4

If cardiac output was expressed as cardiac index the mean value in all 60 patients was 3 24 ± 0 06 l/min/m² In the 40 patients with sinus rhythm the corresponding figures were 3 53 ± 0 13 l/min/m² and in the atrial fibrillation group 2 66 ± 0 14 l/min/m² The difference between the mean values in the latter two groups was highly significant

Stroke volume

The mean value of stroke volume in 27 females with sinus rhythm was 63 4 ± 3 8 ml and in the 13 males with sinus rhythm 84 1 ± 5 5 ml

In the 40 patients with sinus rhythm the stroke volume related to body surface (stroke index) was 41 5 ± 1.8 ml/m²

The quotient between stroke volume and blood volume in the 37 patients with sinus rhythm in whom the blood volume was measured was 13 9 ± 0 6 ml/l. The relation between this quotient and pulse frequency in patients with sinus rhythm and the range in healthy individuals studied at this laboratory (Holmgren et al. 1960) are illustrated in fig 5

Mean pressure in the right atrium

The mean pressure in the right atrium measured in 45 patients was 3 7 ± 0 6 mm Hg In one patient with manifest edema (case 55) the pressure was 24 mm Hg In the rest of the patients the highest value was 10 mm Hg

End-diastolic pressure in the right ventricle

The mean value of the end-diastolic pressure in the right ventricle measured in 59 patients was 4 7 ± 0 5 mm Hg In one patient (case 55) the pressure was 22 mm Hg, in the rest of the patients the highest value was 10 mm Hg. There was no significant difference between the group of patients with sinus rhythm and that with atrial fibrillation.

Pulmonary artery mean pressure

The mean value of the mean pressure in the pulmonary artery in all 60 patients was 34 3 ± 1.8 mm Hg without any significant difference in pressure between the groups of patients with sinus rhythm and with atrial fibrillation.

Pulmonary arterial wedge mean pressure

The mean value of the pulmonary arterial wedge mean pressure in 59 patients was 21 8 ± 0.9 mm Hg The values in patients with sinus rhythm and with atrial fibrillation were not significantly different.

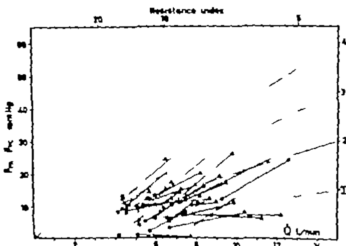


Fig. 7 The relation between pressure drop over peripheral pulmonary vessels ($P_{PA} - P_{PCV}$) and cardiac output ($\dot{Q}$) before operation. Individual symbols as in figure 4. The unbroken thin lines are isoresistance index lines. Upper limit of normal ratios in healthy individuals reaches slightly above isoresistance index line 1 (thin broken line).

cm $^{-5}$. There was no significant difference between the mean values of resistance in the 39 patients with sinus rhythm and the 20 patients with atrial fibrillation. The relation between pressure drop over the peripheral pulmonary vessels ($PA_m - PWP_m$) and cardiac output in all patients and the isoresistance index lines are illustrated in fig. 7.

The resistance in the systemic circulation

In analogy with the calculation above, the index of the resistance in the systemic circulation was calculated in the following way:

$$\frac{(BrA_m - RA_m)\ \text{mm Hg}}{CO\ \text{l/min}} \cdot 80 = R \text{ (dynes sec cm}^{-5}\text{)}$$

(BrA_m = mean pressure in brachial artery, RA_m = mean pressure in right atrium, CO defined as above.)

In 14 patients no right atrial pressure was recorded and end-diastolic pressure in the right ventricle was used instead.

The mean value of resistance in the systemic circulation was in all 60 patients 1312 ± 56 dynes sec cm $^{-5}$.

The corresponding value for the group of 40 patients with sinus rhythm was highly significantly lower than in the group of 20 patients with atrial fibrillation.

Specific ventilation

The mean value of specific ventilation measured as the ratio of ventilation in ml_{BTPS}/min to oxygen consumption ml_{STPD}/min was in 60 patients 33 ± 1. There was no significant difference in mean values between the group of patients with sinus rhythm and that with atrial fibrillation.

Exercise

In almost all patients pressure and flow measurements during exercise at equal loads pre- and postoperatively were used for analysis. If there were several such loads the highest was chosen. Some patients however worked at different loads pre- and postoperatively. In these cases the values from the most equal loads were chosen. In none of these cases was the difference between pre- and postoperative loads greater than 100

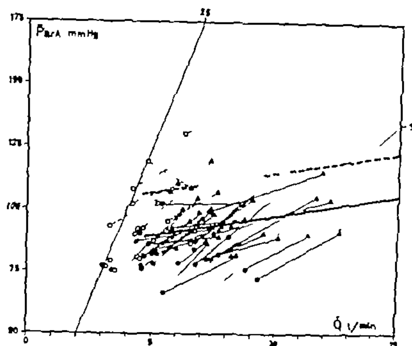


Fig. 6. Mean pressure in the systemic artery ($\bar{P}_{BrA}$) in relation to cardiac output ($\dot{Q}$) at rest and during exercise before operation. *Individual symbols as in figure 4.* Regression line (heavy line) + 2 S_y (broken heavy line) in healthy individuals. The thin lines *are isoresistance index* lines.

Right ventricle work against pressure

Right ventricle work against pressure was calculated according to the usual formula CO l/min 1 055 (PA_m — RV_{end}) mm Hg 13 6 $\frac{1}{1000}$ kpm/min (CO = Cardiac output, PA_m = pulmonary artery mean pressure RV_{end} = end-diastolic pressure in right ventricle) In one patient (case 48) the right ventricular pressure was not recorded and the mean pressure in the right atrium was used instead.

The mean value of right ventricle work against pressure in all 60 patients was 2 3 ± 0 13 kpm/min In the 40 patients with sinus rhythm the corresponding figure was 2 44 ± 0 17 and in the 20 patients with atrial fibrillation 1 82 ± 0 13 kpm/min.

The difference between these two groups was probably significant.

Mean pressure in the systemic artery

The pressure in the systemic circulation was measured in the brachial artery in 58 patients and in the aorta in two patients

The mean value of the mean pressures in 60 patients was 84.2 ± 1 7 mm Hg, the corresponding value in the 40 patients with sinus rhythm 79 5 ± 1 4 mm Hg and in the 20 patients with atrial fibrillation 93 7 ± 1 1 mm Hg. The difference between the two groups was highly significant. The relation between mean pressures in systemic artery and cardiac output in all patients and the regression line and + 2 Sy.x for healthy individuals (Holmgren et al. 1960 and Bevegård et al. 1960) are illustrated in fig 6.

The resistance in the pulmonary circulation

As an index of the resistance (R) in the pulmonary circulation the usual formula was used $\frac{(PA_m - PWP_m) \text{ mm Hg}}{\text{CO l/min}}$ 80 = R (dynes sec cm^{-5}) (PA_m and CO are defined as above PWP_m = pulmonary arterial wedge mean pressure.) The mean value of resistance in 59 patients was 221 ± 30 dynes sec

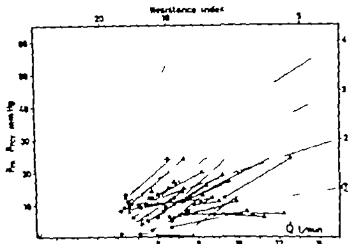


Fig. 7 The relation between pressure drop over peripheral pulmonary vessels ($\bar{P}_{PA} - P_{PCV}$) and cardiac output ($\dot{Q}$) before operation. Individual symbols as in figure 4. The unbroken thin lines are isoresistance index lines. Upper limit of normal variation in healthy individuals reaches slightly above isoresistance index line 1 (thin broken line).

cm⁻⁵ There was no significant difference between the mean values of resistance in the 39 patients with sinus rhythm and the 20 patients with atrial fibrillation. The relation between pressure drop over the peripheral pulmonary vessels ($PA_m - PWP_m$) and cardiac output in all patients and the isoresistance index lines are illustrated in fig. 7

The resistance in the systemic circulation

In analogy with the calculation above, the index of the resistance in the systemic circulation was calculated in the following way

$$\frac{(BrA_m - RA_m) \text{ mm Hg}}{\text{CO l/m}^2} \cdot 80 = R \text{ (dynes sec cm}^{-5})$$

(BrA_m = mean pressure in brachial artery, RA_m = mean pressure in right atrium, CO defined as above)

In 14 patients no right atrial pressure was recorded and end-diastolic pressure in the right ventricle was used instead.

The mean value of resistance in the systemic circulation was in all 60 patients 1312 ± 56 dynes sec cm⁻⁵

The corresponding value for the group of 40 patients with sinus rhythm was highly significantly lower than in the group of 20 patients with atrial fibrillation.

Specific ventilation

The mean value of specific ventilation measured as the ratio of ventilation in ml_{STPD}/min to oxygen consumption ml_{STPD}/min was in 60 patients 33 ± 1. There was no significant difference in mean values between the group of patients with sinus rhythm and that with atrial fibrillation.

Exercise

In almost all patients pressure and flow measurements during exercise at equal loads pre- and postoperatively were used for analysis. If there were several such loads the highest was chosen. Some patients however worked at different loads pre- and postoperatively. In these cases the values from the most equal loads were chosen. In none of these cases was the difference between pre- and postoperative loads greater than 100

Table 18
Oxygen uptake ml $_{STPD}$ /min (x) and cardiac output l/min (y) during exercise
Correlation and regression

	n	$\bar{x}$	$\bar{y}$	r	P	regr equation	S y.x
All patients	40	777	8.2	0.756	<0.001	y = 0.0074x + 2.44	1.32
Sinus rhythm	30	786	8.6	0.821	<0.001	y = 0.0079x + 2.39	—
Atrial fibrillation	10	750	6.9	0.607	0.1—0.05	—	1.21
Normal	27	1467.2	15.36	0.930	<0.001	y = 0.0057x + 7.00	1.36

kpm/min When exercise had been performed only during the preoperative catheterization, the values measured at the highest load were chosen.

Cardiac output

40 patients were studied during exercise. 30 patients had sinus rhythm and 10 atrial fibrillation. The loads varied between 75 kpm/min and 400 kpm/min half of them between 100—300 kpm/min. The range of oxygen uptake was 369—1212 ml/min with half of the values between 628—920 ml/min. The range of cardiac output was 2.9—12.2 l/min with half of the values between 6.8—9.0 l/min.

There was a highly significant positive correlation between oxygen uptake and cardiac output (r = 0.756) (Table 18 and figure 4). The regression equation with cardiac output in l/min as dependent variable and oxygen uptake in ml $_{STPD}$ as independent was Y = 0.007 x + 2.44

If only values from the 32 patients with sinus rhythm were used, the correlation coefficient between oxygen uptake and cardiac output was higher (r = 0.821) and the regression equation with the same definitions as above was Y = 0.008 x + 2.39 There was no significant correlation between the corresponding variables in the ten cases of atrial fibrillation. At the mean oxygen uptake 750 ml/min, of the atrial fibrillation group the cardiac output in the sinus rhythm group was 1.4 l/min higher than in the atrial fibrillation group, a non-significant difference

Arterio venous oxygen difference

The A—V oxygen differences were widely scattered with a tendency to higher A—V oxygen differences in patients with larger oxygen uptakes (fig 3) Most of the patients with atrial fibrillation had higher A—V oxygen differences than those with sinus rhythm within corresponding ranges of oxygen uptake, in accordance with the lower cardiac output of the patients with atrial fibrillation.

The range of A—V oxygen difference in the 30 patients with sinus rhythm was 65.3—125.6 ml/l with an interquartile interval of 81.6—103.0 ml/l, and in the 10 patients with atrial fibrillation the range was 86.7—148.8 ml/l with half of the values between 88.8—128.5 ml/l.

Stroke volume

In the 30 studied patients with sinus rhythm the stroke volume decreased during exercise compared with the value at rest. The mean difference 7.2 ml, was highly significant,

and the mean quotient between the stroke volume during exercise and the value at rest was 0.91 ± 0.02.

Mean pressure in pulmonary artery

There was no significant correlation between cardiac output and mean pressure in the pulmonary artery in the whole material of 30 patients (r = —0.30) After elimination of the 10 cases with atrial fibrillation, however the correlation (= —0 45) between the same variables was probably significant. The regression of mean pressure on cardiac output in the sinus rhythm group was compared with mean values of corresponding variables in the fibrillation group. At the cardiac output of 6.9 l/min the mean pressure was 10 2 mm Hg higher in the sinus rhythm group. The difference was probably significant

Pulm nary arterial wedge mean pre sure

The correlation between pulmonary arterial wedge mean pressure and cardiac output was not significant either in the whole group of 37 patients or in the atrial fibrillation or sinus rhythm groups. The range of pressures was 24—53 mm Hg with an interquartile interval of 34—43 mm Hg and the mean pressure and dispersion of the pressures were about equal in the sinus rhythm and atrial fibrillation groups.

In all patients except two (cases 9 and 60) the pulmonary arterial pressure and pulmonary arterial wedge pressure were recorded during exercise at the same loads

Mean pre sure in brachial artery

No acceptable record of brachial artery pressure could be obtained in one patient (case 2) on account of the damped curve. The reported mean pressures in pulmonary artery and in brachial artery were recorded during exercise at the same loads.

No significant correlation between cardiac output and brachial arterial mean pressure was found either in the whole group of 39 patients or in the sinus rhythm or atrial fibrillation groups (fig. 6)

The range of pressures in all patients was 79—146 mm Hg with half of the values between 90 and 105 mm Hg. The corresponding range in the 10 patients with atrial fibrillation was 91—146 mm Hg with an interquartile interval of 100—120 mm Hg The corresponding figures for the sinus rhythm group were 79—115 and 89—104 mm Hg respectively If only pressures with corresponding cardiac outputs within the range of the fibrillation cases (5 7—8 7 l/min) were compared, then the pressures in the trial fibrillation rhythm group were highly significantly[1]) higher than in the other group

The esistance index in the pulmonary circulatio

In 26 of the 30 patients with sinus rhythm in whom both pulmonary arterial wedge pressure and pulmonary arterial pressure could be measured, there was a highly significant increase in pulmonary vascular resistance index during work compared with the values at rest (fig 7) The mean difference was 32 dynes sec cm $^{-5}$ In the 10 patients with atrial fibrillation the change in resistance index compared with the resting values was not significant. In 7 of the 26 patients with sinus rhythm and 4 of the 10 patients with atrial fibrillation there was a decrease of pulmonary resistance during exercise. Of the 4 patients with resistance values of more than 200 dynes sec cm^{-5} an increase was found in tw of them and a decrease in the other two

[1]) T o-sample ranks test

The resistance index in the systemic circulation

As right atrial pressures were not measured during exercise and right ventricle pressures not in all patients, a resistance index was calculated in 39 cases from systemic mean pressure and cardiac output only. These values were compared with values calculated in the same way from measurements at rest.

In the 29 patients with sinus rhythm and also in the 10 patients with atrial fibrillation there were highly significantly lower values of indices of peripheral resistance during exercise than at rest (fig. 6). The mean decrease in resistance index in the patients with sinus rhythm was 2.5 units corresponding to 200 dynes sec cm^{-5} and in those with atrial fibrillation 5 units corresponding to 400 dynes sec cm^{-5}. Only one patient (case 36) showed an increase in peripheral resistance during exercise. This patient had also a high pulmonary artery mean pressure at rest, 63 mm Hg, with an increase up to 91 mm Hg during exercise at a small load, and she had a small cardiac output both at rest and during work. Her peripheral resistance during work was 2456 dynes sec cm^{-5}. In the rest of the material there was no resistance during work above 1600 dynes sec cm^{-5}. The patients in the atrial fibrillation group who had the highest resistance values at rest had the greatest decrease of these values during work.

Ventilation

The mean value of oxygen uptake during exercise in 39 patients was 768 ml_{STPD}/min and the mean value of ventilation 23.8 l_{BTPS}/min.

The correlation between these two variables was highly significant ($r = 0.688$) and the equation of linear regression of ventilation (y) on oxygen uptake (x) was $Y = 0.0217\,x + 7.2$ (table 19). The correlation between the same two variables was highly significant both in the group of patients with sinus rhythm and in that with atrial fibrillation. The difference between the corresponding two regression lines was not significant.

Comments on methods

Reproducibility

Duplicate estimations of cardiac output calculated from two sampling periods usually at only some minutes interval were performed on 27 patients at rest. No systematic difference between the first and second sample period was found. The standard error of a single estimation was 7.6 % in agreement with other studies of mitral stenosis (Holmgren et al., 1958, Werkö 1964) in which the corresponding values were 8.3 % and 7 % respectively. Duplicate estimations during work were not possible in the present material without selection of the cases.

Table 19

Oxygen uptake ml_{STPD}/min (x) and ventilation l_{BTPS}/min (y) during exercise. Correlation and regression

	n	$\bar{x}$	$\bar{y}$	r	P	regr. equation	$S_{y.x}$
All patients	39	768.1	23.83	0.688	<0.001	$y = 0.02171x + 7.15$	4.39
Sinus rhythm	29	774.4	24.13	0.655	<0.001	$y = 0.01944x + 9.07$	4.82
Atrial fibrillation	10	749.8	22.95	0.824	<0.001	$y = 0.03211x + 1.12$	3.99

Holmgren & Pernow (1960) showed in 27 cases, 22 of which with heart or lung disease, that the reproducibility of cardiac output by the direct Fick method was better during muscular work than at rest. The coefficient of variation of stroke volume during exercise in the same study was 6.8 %.

The mean deviation of + 20.9 % from the measured oxygen uptake during "basal" conditions agreed well with reported figures from most heart catheterizations (Karlöf & Jonsson, 1963).

Correlations

In evaluation of some of the correlations and regressions, common elements in the variables must of course be taken into consideration, for example in the relation between oxygen uptake and cardiac output and between oxygen uptake and ventilation.

Stroke volume

Values of stroke volume were calculated only in cases with sinus rhythm because a calculated mean value of stroke volume in fibrillating cases is much too dependent on the degree of irregularity in heart rhythm to be useful in comparisons.

Resistance index

An error in calculation of the systemic resistance index during work is introduced by using only the mean pressure in the brachial artery instead of the difference between this pressure and the right atrial pressure. To estimate this error the end-diastolic pressure in the right ventricle may be substituted for the mean atrial pressure as according to most studies, these two pressures usually do not deviate markedly from one another. The end-diastolic pressure in the right ventricle was measured during exercise in 34 patients and, with a few exceptions, at the same loads as reported above. The mean value of these pressures was + 11.4 mm Hg and the range 0—20 mm Hg. As the mean value of the brachial arterial mean pressure on exercise was 98.9 mm Hg (range 79—146 mm Hg) the systematic error introduced in the resistance mean value in the whole group can be estimated at about + 10 %. The index was calculated in the same way in other studies of mitral stenosis (Gorlin & Gorlin, 1951 and Eliasch, 1952).

Resistance in pulmonary circulation

The pulmonary vascular resistance index was, as usual, calculated as a quotient between the mean values of two integrated functions, namely the rate of change with time of pressure gradient and of flow respectively. Correctly it should be a mean value of one function, the integral of the rate of change with time of the quotient between pressure gradient and flow (Harris & Heath, 1962). A similar error in flow measurements was discussed above. The magnitude of the influence of these errors on resistance values is not known. Many authors have found evidence of a pulsatile flow in the pulmonary capillary vessels in normal men (Lee & Du Bois, 1955, Chrispin & Steiner, 1964). Important phase differences between pressure and flow may thus be possible. The relative importance of these phase differences is said to be less in patients with increased pressures in the pulmonary artery (Harris & Heath, 1962).

The relation between pressure gradient and flow in the pulmonary circulation in supine position was shown to vary in healthy men during exercise and these values deviate usually from those at rest (Bevegård et al., 1960, Harris & Heath, 1962, Holmgren et al., 1962, Granath et al., 1964). Such varia-

tions were also observed in materials of mitral stenosis (Eliasch 1952 Holmgren et al., 1958)

If a critical closure pressure exists in the pulmonary capillaries there will be varying relations between pressures and flows also if this relationship is linear Both intra and interindividual comparisons between resistance values should thus be evaluated in relation to the absolute values of pressures and flows

Pulmonary arterial wedge pressure

Since the introduction of the technique of recording pulmonary venous pressure by placing a catheter in the pulmonary artery wedge position (Lagerlöf & Werkö 1949 Hellems et al., 1949) the validity of this method has been much discussed Most studies during the last years have shown that if uncharacteristic curves are dismissed, pressure curves from the left atrium and from pulmonary arterial wedge position agree very well both in configuration and in pressure level (see Werkö 1964)

Comments on results at rest

Arterio venous oxygen difference and cardiac output

Figure 3 shows that most of the patients with atrial fibrillation had an A—V oxygen difference at rest above normal variation and those with sinus rhythm within the same variation.

The mean values of arterio-venous oxygen difference and of cardiac index in the whole group agreed well with those in other studies of mitral stenosis, and best with values for patients in groups II—III according to the classification of N Y H A. (Eliasch, 1952, Bishop & Wade, 1963)

There was a highly significant difference in elevation between the regression of cardiac output on oxygen uptake in the patients with sinus rhythm and in a normal material studied at this laboratory (Holmgren et al., 1960 Bevegård et al., 1960) The difference between adjusted means was 1.26 l/min with the normal individuals represented by the upper regression line

A difference in cardiac output between patients with sinus rhythm and with atrial fibrillation was found by Wade & Bishop (1962) in 181 patients with mitral stenosis, 71 of whom had atrial fibrillation. The difference was significant The mean values, 3 02 and 2.31 l/min/m^2 were somewhat lower than in the present material. A significant increase in cardiac output after conversion of atrial fibrillation to sinus rhythm was shown by Gilbert et al (1963) in a study of 11 patients, 9 of whom had mitral stenosis, and by Broch & Müller (1957) in 20 patients, 11 of whom with mitral stenosis. Selzer (1960) found in 13 matched pairs of patients with mitral stenosis selected from a material of 67 patients that the mean value of cardiac output in patients with sinus rhythm was significantly higher 0 54 l/min/m^2 than in patients with atrial fibrillation

Stroke volume

In a study at his laboratory (Holmgren et al., 1960) the lower limit of the range of stroke volume in individuals without heart disease was in a group of males 83 ml and in a group of females 75 ml The mean values of stroke volume in corresponding groups in the present study were thus lower than or at the lower limit of those ranges The mean values of the quotients between stroke volume and blood volume in different groups were in the same study between 1 92 % and 2.21 % and the normal range of these quotients is illustrated

in fig. 5 The figure shows that about half of the patients in the present study had quotients below the lower limit of normal variation.

The mean value of stroke volume in the females in the present study was not significantly different from the corresponding value, 61.9 ml, in 13 females with pure mitral stenosis in a study by Holmgren et al. (1958)

Pulmonary arterial mean pressure
pulmonary arterial wedge mean pressure

The mean values of the pulmonary arterial mean pressure and the pulmonary arterial wedge mean pressure were above the upper limit of normal variation (Holmgren et al., 1960 Granath, Jonsson, Strandell, 1964) They were similar to those found by Eliasch (1952) in clinical group III according to N.Y.H.A., 34 and 20 mm Hg respectively and also to those of the same group studied by Werko (1964) but the pressures in the present material were lower than in the 32 patients selected for valvulotomy reported by Wade & Bishop (1962)

Pulmonary vascular resistance

The mean value of vascular resistance in the pulmonary circulation was slightly above the upper normal limit which is set between 100—200 dynes sec cm⁻⁵ in different studies (Wood, 1956 Holmgren et al., 1960 Harris & Heath, 1962) The resistance values corresponded very well with Wood' material of mitral stenosis in which 80 % of the cases had normal or only slightly raised pulmonary resistance and 7.5 % values between 800—2004 dynes sec cm⁻⁵ In the present material the corresponding value of the latter incidence was 6.7 % and 4 patients had resistance values above 500 and one above 800 dynes sec cm⁻⁵

Right ventricle work against pressure

In 16 patients, mostly with mitral stenosis, studied before and after restoration of sinus rhythm, Broch & Müller (1957) found a probably significantly higher mean value of right ventricle work against pressure in sinus rhythm than in atrial fibrillation. In the present material the corresponding mean difference was not significant. The patients of Broch & Müller had considerably lower pulmonary arterial pressures and the values of right ventricle work were lower than in the present material. The mean value of right ventricle work against pressure in 8 women and 3 men with mitral stenosis and sinus rhythm, 2.07 kpm/min, studied by Thomasson et al. (1959) agreed well with the values in the present material.

The mean value related to body surface in the sinus rhythm group in the present material, 1.5 kpm/min/m² was somewhat lower than in that group in the material of Gorlin & Gorlin (1951) 1.9 kpm/min/m²

Resistance indices

The mean value of systemic resistance index was higher than the upper limit of normal variation (Varnauskas, 1955)

A higher mean value of systemic resistance in an atrial fibrillation than in a sinus rhythm group was found by Gorlin & Gorlin (1951) in patients with mitral stenosis. The mean values from 16 patients were higher than in the present material in both groups of heart rhythm. Right atrial mean pressure was excluded in the calculations of resistance values in the present material before this comparison. The patients of Gorlin & Gorlin were in worse clinical condition than in the present study The mean difference in resistance values between the two materials was almost 400 dynes sec cm⁻⁵ in the sinus rhythm groups. Also in the material

studied before and after valvulotomy by Eliasch (1952) the preoperative mean value in the patients with sinus rhythm, 1543 dynes sec cm^{-5} was higher than in the present study

Specific ventilation

The mean value of specific ventilation was within the upper normal range 28 ± 3 (Rossier et al., 1960) and was similar to that in 28 patients in clinical groups I and II according to N.Y.H.A. classification in a material of mitral stenosis analysed by Frank et al. (1953) by closed spirometry. For comparison, the values of ventilatory equivalent in that study were converted to specific ventilation by using a factor of 12.11. The mean value was about 3 units higher than in the present material.

In a material studied by spirography before and after commissurotomy by Dogliotti et al. (1959) the corresponding value 46.5 units was much higher

Comments on results during exercise

In most studies of patients with mitral stenosis exercising in supine position, the work loads have been smaller than in the present material. Thus in the material studied by Eliasch (1952) the greatest value of oxygen uptake was less than 400 ml/min/m^2 body weight. The corresponding mean value in the present material was 459 ml/min/m^2 body weight.

In the 52 patients studied during exercise by Werkö (1964) the highest mean value, not related to body surface, in the different groups was 510 ml/min.

The preoperative exercise mean value of oxygen uptake, 443 ml/min/m^2 in the material of Wade & Bishop (1962) was similar to that in the present material.

Arterio-venous oxygen difference and cardiac output

The A—V oxygen differences of all patients with atrial fibrillation and of most with sinus rhythm were outside two standard deviations during exercise (fig. 3)

There was a highly significant difference in elevation of the linear regression of cardiac output on oxygen uptake during exercise in all patients compared with a normal material of 27 individuals studied at this laboratory (Holmgren et al., 1960 Bevegård et al. 1960) (Table 18). In the linear regression of cardiac output (y) on oxygen uptake (x) in the patients with sinus rhythm the sample standard deviation from the regression line was almost the same as in the normal material. The difference in elevation between the latter two regression lines (difference between adjusted means) was about 4.5 l/min. Bishop & Wade (1962) also found in a material analysed in a somewhat different way a greater increment of cardiac index with increasing oxygen uptake in all groups of patients with sinus rhythm compared with those with atrial fibrillation if the average increase of cardiac output was adjusted to a common increase in oxygen uptake, but the differences were not significant.

Stroke volume

In a study by Holmgren et al. (1958) the relative mean decrease of stroke volume during work compared with that at rest was 13.7 % in 17 patients with sinus rhythm and pure mitral stenosis and working at similar loads to those in the present study. This decrease was somewhat more marked than in the present study

Relations between cardiac output and pulmonary artery pressure

Bishop & Wade (1962) found a significant negative correlation (—0.41) between cardiac index and pulmonary arterial mean pressure during exercise in different patients with sinus rhythm. The corresponding correlation coefficient in the present material was probably significant, —0.45. Only a small part of the whole variation in pulmonary arterial mean pressures in different patients was thus caused by such a regression in either material.

Pulmonary arterial wedge pressure

The mean and range of pulmonary arterial wedge mean pressures during exercise in the present material agreed very well with corresponding values reported by Gorlin & Gorlin (1951) 40 mm and 28—51 mm Hg respectively but the oxygen uptakes were considerably greater in the present material.

In most similar studies both oxygen uptakes and cardiac outputs during exercise are considerably lower than in the present material and are thus not comparable.

Resistance indices

The varying changes of resistance during exercise are consistent with findings in most studies of mitral stenosis (Eliasch, 1952, Werkö, 1964). The incidence of different kinds of changes varies in different materials. According to Harris & Heath (1962) a rise of the values during exercise is most common, while Gorlin & Gorlin (1951) found no significant mean change of resistance during exercise.

Fig. 6 shows that the peripheral vascular resistance index during exercise was within the normal range in all except one patient.

Ventilation

The values of ventilation during exercise seem to agree well with those in patients with mitral stenosis and with the best postoperative results studied by Donald et al. (1957). In that study the mean ventilation before operation was 19.45 l/min/m² and the mean oxygen uptake 462 ml/min/m² and the ventilation was compared with a predicted normal mean value of 8.4 l/min/m² for normal subjects.

OPERATIVE TECHNIQUE, VALVULAR ANATOMY AND GROUPS

All patients were operated upon by the closed operative technique with surgical approach through the fifth intercostal space on the left side. Through the incision in the left appendage blood was allowed to flow out from the atrium for a while to eliminate detached clots The mitral valve was palpated for evaluation of valvular anatomy and regurgitation In all cases digital valvulotomy was done or tried primarily Only when the valvulotomy was difficult to perform with the finger or the split was judged to be unsatisfactory i e the mitral orifice was estimated to be less than two fingers was the operation performed or completed with Dubost s dilator inserted via the ventricle. If possible the ostium was then dilated to a width of two fingers In some cases however when a moderate regurgitation was felt after an unsatisfactory digital valvulotomy or if extensive and marked calcification or fibrosis of the valves were palpated, the dilator was not used. In these cases an eventual improvement in result obtained with the instrument was judged not to outweigh the risks of a large regurgitation. These indications for instrumental valvulotomy were the same for all patients in the present material

After the split of the valve the surgeon estimated, again by palpation, the size of the mitral orifice and the existence or not of regurgitation. In 30 patients digital valvulotomy was performed in the other 30 patients the split was made or completed with the dilator In the present material the relation between numbers of patients operated by the two methods was about the same during each of the four years of the study

As already noted, all operations were performed by the same surgical team of the Department of Thoracic Surgery Karolinska sjukhuset (Head Professor Crafoord)

The grouping of the present material (Table 20) was based mainly on results of palpation, noted by the surgeon, in respect of tightness of the valve before valvulotomy the adequacy of split, the existence and degree of fibrous and calcification of the valves, and the existence or not of palpable regurgitation in the left atrium. The latter finding was evaluated in conjunction with the phonocardiogram.

Tightness of the mitral valve before valvulotomy

A mitral orifice that did not admit the end phalanx of the surgeon s finger was defined as tight (group 1) a mitral orifice that did admit the phalanx as less tight (group 2)

Forty-two patients were classified in group 1 and 17 in group 2. In one patient there was no note about the tightness of the valve (case 48) No patient was judged to have a mitral orifice larger than two fingers.

Palpable regurgitation before valvulotomy

In 25 cases the surgeon palpated a regurgitation in the left atrium, in 33 cases he did not. In two cases there was no operative record of the existence or absence of regurgitation Even if the surgeon described the results of palpation as a very slight insufficiency possibly a slight insufficiency or if he used other terms to describe very small or scarcely palpable

Table 20

Criteria for grouping the material and number of patients in different group

	Groups	Number of patients	Criteria	Comments
Tightness of the mitral valve before commissurotomy evaluated by the surgeon at operation	1	42	Mitral orifice less than the distal phalanx	
	2	17	Mitral orifice that admits the distal phalanx	
Total		59		One patient not grouped (case 41)
Valvular involvement before commissurotomy	A	32	"Pure" mitral stenosis	
	B	28	Combined valvular lesions	
Total		60		All patients grouped
Adequacy of the split of the mitral valve evaluated by the surgeon at operation	S	35	Mitral orifice judged to admit two fingers	
	L	24	Mitral orifice judged not to admit two fingers	
Total		59		One patient not grouped (case 45)
Functional postoperative result evaluated according to the classification of N Y H A.	I	40	Lower group after operation	
	N I	20	Unchanged or higher group after operation	
Total		60		All patients grouped

regurgitation streams, the findings were considered to signify insufficiency. Twenty five cases had mitral insufficiency defined in that way. Only in one patient (case 52) was the insufficiency described as marked. In that case as well, however the mitral rea was judged to be less than the width of tw fingers and the surgeon considered the stenosis to be dominating. To judge from the operation records, no mitral regurgitation was evaluated to be larger than that in the case above. As regards the two cases without any record of insufficiency it may be assumed that if a marked insufficiency

had been palpated such a finding would have been noted

Classification according to valvular lesion

The classification of the patients according to valvular lesion before valvulotomy was based on the existence or absence of palpable insufficiency in the left atrium and on phonocardiographic findings

A patient was considered to have pure mitral stenosis (group A) if no regurgitation was palpated in the left atrium and there was no pansystolic apical murmur indicating mitral insufficiency and no murmurs characteristic of aortic valvular lesion on the phonocardiogram. In one patient (case 2) who was referred to group A, there was no record of the existence or absence of palpable regurgitation, but the valves were pliable and movable and he had no phonocardiographic signs of mitral insufficiency Thirty two patients were classed in group A.

The rest of the patients were classed in group B The latter group consisted of 28 patients 19 of whom were judged to have combined mitral valvular lesions and 9 combined aortic and mitral valvular lesions. The diagnosis of aortic lesion was based on analysis of the phonocardiogram. In two patients the diagnosis of mitral insufficiency was not based on the surgeon s findings. In one (case 39) there was no palpable regurgitation and in the other (case 40) there was no note as to the existence or absence of such regurgitation. The first patient had a pansystolic high-frequency murmur with maximal intensity over the apex, the other had no characteristic murmur but the valves were calcified and scarred and the patient had a large heart volume, sinus rhythm, and a small diastolic mean pressure gradient of the mitral valve, findings that were considered to suggest the existence rather than the absence of some degree of mitral insufficiency

Absence of apical systolic murmur in patients with palpable regurgitation was noted in 15 of the patients

Of the nine patients with combined aortic and mitral valvular lesions three (cases 33 41 62) had signs of both stenosis and insufficiency and six of insufficiency alone in the aortic valves All but one of the nine patients (case 25) had signs of mitral insufficiency

The surgeon s evaluation of fibrosis and calcification

In nine cases the operative records contained no note of fibrosis or calcification of the valves. In the other cases a description of the anatomy of the valves permitted a rough classification of the changes. Patients who had no or only a slight degree of fibrosis and/or calcification of the valves were referred to group I, and patients who had these changes in a moderate or marked degree to group II. Twenty-two patients were classed in group I and 29 in group II.

Adequacy of the split of the valve

In 35 cases the surgeon considered the split to be satisfactory i.e the valve was estimated to admit two fingers. These cases were classed in group S.

In 24 cases the split was judged to be less satisfactory i.e. the valve was considered not to admit two fingers and these patients were classed in group L. In one patient (case 45) there was no note of the estimated area after the split.

The surgeon could palpate a mitral insufficiency after the split in 35 cases, in 20 cases no such insufficiency was palpated In five cases no report was made on the existence or absence of palpated insufficiency after the split.

Comments on findings at operation

The evaluation of mitral area by palpating the mitral valve at operation is a rough method. Also when the mitral area is measured at autopsy the values vary markedly with the technique of measurement.

In an investigation by Richter (1963) the mitral area was measured in different ways and the highest value was 65 % above the lowest. The calculated values of the area differed according to whether the measurement was made after stretching of the valve or not, and in some degree according to whether the area was measured directly or approximated to the area of a circle calculated from the circumference. The valve was fish-mouthed in Richter's case and there was only slight calcification. A thoracic surgeon palpated the valve and estimated the area. The estimation agreed well with the approximate value of the circular area calculated from the circumference measured on paraffin cast.

Thus there must be great difficulties in estimating the area of the mitral valve by palpation at operation. The accuracy of the method seems to depend on the degree of fibrosis and calcification, the shape of the area, the degree of stretching when the surgeon is palpating the valve and the dimensions of the surgeon's finger.

Too detailed a classification based on mitral area estimated at operation may thus be assumed to be of small value.

It might be of interest to compare the effect of digital and instrumental valvulotomy and such comparisons have been reported. A prerequisite for the comparison, however is that every patient has been operated upon by only one of the two methods and that the method was chosen at random. In the present material, as instrumental valvulotomy was a complementary method, any differences in postoperative results between the two methods are probably due mainly to differences in valvular anatomy

The evaluation of insufficiency

The accuracy in evaluating the existence or absence of a regurgitation by palpation at operation is difficult to assess. The existence or not of mitral regurgitation evaluated by the surgeon at operation has been used as reference in most published studies of methods for the diagnosis of mitral insufficiency. A regurgitating blood flow through insufficiently closed valves is, among other things, a function of pressure gradient and heart frequency both of which are generally changed at operation. It seems probable, however, that the lack of palpable regurgitation at operation excludes a mitral insufficiency of functional importance provided that there are not too great changes in the hemodynamics during operation.

In the present material there was only one patient who did not have a palpable insufficiency at operation but had a high-frequency systolic murmur with maximum over the apex thus a mitral insufficiency diagnosed on the phonocardiogram was in general confirmed at operation.

The difficulty in evaluating the degree of mitral insufficiency by palpation must be even greater than in evaluating the existence or absence of such an insufficiency The hemodynamics during operation are changed and the sensation of the regurgitant blood stream in the palpating finger is probably better correlated to velocity of regurgitating blood stream than to total regurgitating blood volume during systole.

The present material was not classified with regard to degree of mitral insufficiency

Table 21

	Palpable regurgitation	No palpable regurgitation	Total
Group 1	18	24	42
Group 2	7	8	15
Total	25	32	57

Table 22

	Degree of calcification		Total
	I	II	
Group S	12	16	28
Group L	10	12	22
Total	2_	28	50

Table 23

	Degree of calcification		Total
	I	II	
Palpable regurgitation	6	17	23
No palpable regurgitation	16	12	28
Total	22	29	51

Table 24

	Degree of calcification		Total
	I	II	
Digital valvulotomy	13	13	26
Instrumental valvulotomy	9	16	25
Total	22	29	51

Comparisons between groups

The incidences of patients with palpable mitral regurgitation before valvulotomy in groups 1 and 2 did not deviate significantly[1]) from the incidence for the whole material (Table 21)

As regards calcification and/or fibrosis of the valves the incidence of patients with moderate or marked changes in groups S and L did not significantly[1]) deviate from that for the whole material (Table 22)

Stewart & Glenn (1959) obtained good splits of the valves in only half as high a percentage of patients with marked calcification of the mitral valves as in those without calcification. The different results in their and in the present material may be due to different definitions of the degrees of calcification and of good splits, and also to different surgical technique

In the groups of patients with moderate or marked fibrosis and/or calcifications of mitral valves there was a probably significantly higher incidence of mitral regurgitation before valvulotomy than for the whole material (Chi square = 4.8) (Table 23) This finding was in accordance with that of Dack et al (1960) who found a doubled incidence of mitral apical systolic murmurs in patients with calcification of the valves compared with those without such changes.

Instrumental valvulotomy was performed somewhat less often than digital valvulotomy in the patients with no or slight calcification and fibrosis of the valves, and somewhat more often in the patients with moderate or marked changes of the valves but the deviation from the frequency for the whole material was not significant (Table 24)

) Chi-square tests.

PREOPERATIVE FINDINGS AND SOME RELATIONS

A great number of combined clinical and hemodynamic studies on patients with mitral stenosis have been reported during the last ten years. It is beyond the scope of this study to deal extensively with the correlations between preoperative findings in the present material. Only some relations that seem to be of special interest will be commented on.

Myocardial insufficiency

For evaluation of postoperative results it is important to know how many of the patients had signs of myocardial insufficiency during the preoperative examinations. There was only one patient with manifest signs of congestive heart failure (case 55). This patient had a very high pulmonary resistance, the highest values of right atrial pressure and heart volume in the whole material, and he also had edema of moderate degree in the legs.

Fleming & Wood (1959) analysed 24 cases with myocardial dysfunction selected from 750 patients with mitral stenosis. All of them had atrial fibrillation, low cardiac output and normal or only slightly elevated left atrial pressure and most were considered to have signs of left heart failure. The patients with high pulmonary resistance had been eliminated from that study. In the present material there was only one patient (case 59) who seemed to fit this description. This patient had had atrial fibrillation since 6 years before the heart operation. He was 50 years of age at operation and had small cardiac output, a pulmonary arterial wedge mean pressure within normal limits and very large heart. At operation the stenosis was found to be of only moderate degree. The patient had a pansystolic murmur over the apex but the regurgitation was hardly felt by the surgeon.

The patients with mitral stenosis and myocardial insufficiency studied by Harvey et al. (1955) had in common a normal or slightly increased pulmonary artery pressure at rest and a low cardiac output. In the present material there was only one patient (case 45) with pulmonary artery mean pressure below 25 mm Hg and with a cardiac output below two standard deviations from the regression line for the normal material studied at this laboratory. In this case the surgeon found a very tight stenosis at the operation.

Cases 55 and 59 were the only ones who had heart volumes larger than 2000 ml, the former 2950 ml and the latter 2760 ml.

Differences between groups 1 and 2

Some relations between the tightness of the mitral ostium and the results of some investigations will be commented upon (Table 25).

Electrocardiography

Of electrocardiographic findings there were differences of any significance between groups 1 and 2 only in P vectors. The mean value of the mean P vector in the frontal plane was 4 degrees higher (more to the right) in group 1 than in group 2. The

Table 25

Differences of some significance between tight (group 1) and less tight stenosis (group 2)

Preoperative values

			n	$\bar{x}$	ε	$\bar{d}$	ε_d	P
Ecg	P vector in frontal plane degrees	group 1	25	49.0	4.0	24.4	11.2	0.01—0.05
		group 2	7	24.6	16.2			
Maximum work intensity	Sitting kpm/min	group 1	29	299	19	116	36	0.001—0.010
		group 2	12	415	41			
	Supine kpm/min	group 1	29	170	16	97	38	0.01—0.05
		group 2	10	267	44			
Right heart catheterization	A—V oxygen difference ml/l	group 1 sinus rhythm	28	48.2	2.4	10.1	4.0	0.01—0.05
		group 2	11	38.1	2.1			
	Stroke volume / Blood volume ml/l	group 1 sinus rhythm	26	13.1	0.7	2.9	1.2	0.01—0.05
		group 2	11	16.0	1.1			
	Stroke volume / Bwt ml/kg	group 1 sinus rhythm	38	37.6	1.9	12.4	3.5	0.01—0.001
		group 2	11	50.0	2.9			
	Pulmonary artery mean pressur mm Hg	gro p 1	42	37.9	2.3	—11.2	3.2	0.01—0.001
		group 2	17	26.6				
	Pulmonary wedge mean pressure mm Hg	group 1	41	23.8	1.0	—6.0	1.7	<0.001
		group 2	17	17.8	1.2			

difference was probably significant. The mean QRS vector in the frontal plane was higher in group 1 than in group 2 but the difference between the groups was not significant. Seven of the eight patients with electrocardiographic recordings of right ventricular hypertrophy type belonged to group 1

Exercise

In group 2 there was a significantly higher mean value of maximal work intensity of the women both in sitting and in supine position than in group 1. In the men there was no such difference of any significance.

Spirometry

There were no significant differences between the mean spirometric values of the two groups.

Heart catheterization

The mean value of the arterio-venous oxygen differences at rest was higher in group 1 than in group 2 in patients with sinus rhythm. The difference was probably significant. In the patients with atrial fibrillation there was no significant difference between the two groups.

The correlation between cardiac output and oxygen uptake at rest was insignificant in patients with atrial fibrillation both in groups 1 and 2, but significant in patients with sinus rhythm in group 1, not in group 2. The mean value of cardiac output in patients with sinus rhythm in group 2 was 1.6 l/min larger than in group 1 at an oxygen uptake of 262 ml/min, which was the mean value of oxygen uptake in group 2, a highly significant difference. There was no difference in cardiac output of any significance between groups 1 and 2 in patients with atrial fibrillation.

In patients with sinus rhythm the stroke volume related both to blood volume and to body weight was smaller in group 1 than in group 2; the differences were probably significant and significant respectively. The stroke volume at work was significantly smaller than at rest in group 1; in group 2 there was no such significant difference.

No significant difference between the two groups with regard to work of the right ventricle against pressure was found.

There was no significant difference either in pulmonary or in peripheral resistance between the two groups.

The pulmonary artery mean pressure was significantly higher and the pulmonary artery wedge mean pressure highly significantly higher in group 1 than in group 2.

The specific ventilation was not significantly different in the two groups at rest.

To summarize — there was good agreement between the surgeon's opinion of the largeness of the ostium before the splitting of the valves and many hemodynamic and some other findings at the preoperative examination. Compared with patients with less tight mitral stenosis (group 2) those with tighter mitral stenosis (group 1) had higher pressures in the pulmonary circulation. In patients with sinus rhythm in the latter group there was a smaller cardiac output and a smaller stroke volume and the women had lower maximal work intensity.

Calcification of the mitral valves

Absence or diminution in intensity of opening snap is related to calcification of the mitral valves in many studies (Mounsey 1953, Wood 1954). In the present material 6 patients of 8 who had no opening snap had calcification and/or fibrosis of moderate or marked degree.

Differences between groups A and B

The relations between some preoperative results and the groupings into pure mitral stenosis (group A) and combined valvular lesions (group B) will be commented upon.

On the phonocardiograms there were no differences in Q—1 or OS—2 interval between these groups.

5

Nor were there any significant differences between the same groups in respect of QRS vector in the frontal plane or of CR_7—CR_2 net area.

No mean difference of any significance between groups A and B was found in maximal work intensity or in $\frac{W_6}{\Delta f}$ in sitting or in supine position, either in males or in females.

No significant differences were found in pulmonary artery mean pressure or in pulmonary wedge mean pressure between the two groups, either in patients with sinus rhythm or in those with atrial fibrillation.

Thus in the present selected material of predominating mitral stenosis the existence or not of combined valvular lesions did not seem to have significantly influenced the preoperative values reported above.

Of the 70 consecutively operated patients who were investigated before heart operation according to the criteria for selection of patients in the present material, four died during the whole follow-up period. Also the 10 patients not investigated after operation are thus included in this group.

Early postoperative mortality

No patient died during or immediately fter the operation. One patient (case 63) female, 58 years of age, died after increasing signs of heart insufficiency 15 days after the operation. She had a pulmonary wedge mean pressure of 21 mm Hg and high value of pulmonary resistance (815 dynes sec cm 5) at the preoperative catheterization. At operation a tight mitral stenosis with fibrous valves was found which necessitated splitting with a dilator. At autopsy the mitral ostium admitted two fingers and some of the chordae tendineae had loosened from their attachment at the dorsal valve. A great incompetence of the valves due to rupture of the chordae tendineae thus seemed to have caused the death of the patient.

Late postoperative mortality

One patient (case 61) a male, 49 years of ge, died 19 months after operation. The preoperative examination of this patient showed signs of a tight mitral stenosis and this was confirmed at the operation which was performed without complications.

During the first six postoperative months he had less symptoms from the heart than before operation after that time he suffered from increasing dyspnea. The patient was an alcoholic. He was admitted unconscious to hospital on the same day as he died in fever and signs of pulmonary edema. The autopsy showed calcified mitral ostium which admitted one finger and a half. The patient had had atrial fibrillation for some years before the heart operation on admission to hospital before his death he had a very rapid ventricular frequency.

The last two of the four deaths (cases 62 and 72) are included in the present study as both patients were investigated at this hospital before their final deterioration. One of them (case 72) female, 42 years of age at operation, died 3 years and 3 months after operation on account f bacterial endocarditis. A pure tight mitral stenosis was found at operation which was performed without complications. Before the operation she had rheumatic symptoms and somewhat increased antistreptolysin titer. After operation she had several periods with symptoms from the joints, fever and elevated antistreptolysin titers, and was treated several times with antibiotics and steroids. A recatheterization 3 months before the patient died showed a good hemodynamic result of the operation with pressures in the pulmonary circulation, cardiac output and stroke volume within normal limits. In the last two years the patient had been continuously treated with steroids, and different types of antibiotics had been tried without durable effect on the fever. When the patient died, she had had

Nor were there any significant differences between the same groups in respect of QRS vector in the frontal plane or of CR_7—CR_2 net area.

No mean difference of any significance between groups A and B was found in maximal work intensity or in $\frac{W_6}{\Delta f}$ in sitting or in supine position, either in males or in females.

No significant differences were found in pulmonary artery mean pressure or in pulmonary wedge mean pressure between the two groups, either in patients with sinus rhythm or in those with atrial fibrillation.

Thus in the present selected material of predominating mitral stenosis the existence or not of combined valvular lesions did not seem to have significantly influenced the preoperative values reported above.

Anamnestic data

Patient's opinion of the result of operation

Four patients (cases 12, 19, 45, 57) considered themselves more incapacitated at the time of postoperative investigation than before operation. Three of them had increased exertional dyspnea, one of these (case 12) marked dyspnea also at rest, and the fourth patient had had periods with edema in her legs of slight to moderate degree. Two patients considered their heart symptoms to be unchanged. Fifty-two of the 58 patients who were alive after the follow-up period felt improved after the operation.

Change of heart rhythm after operation

Seven patients had developed atrial fibrillation since the preoperative investigation and one of them (case 34) already before heart operation. Two patients reverted to sinus rhythm on quinidine after the heart operation. At postoperative investigation 35 patients had sinus rhythm and 25 atrial fibrillation. One of those with sinus rhythm (case 4) had atrial fibrillation during heart catheterization and reverted to sinus rhythm the day after.

Two patients (case 9, 56) had had several short periods of atrial fibrillation, one patient (case 50) had had one only. Another patient (case 27) with atrial fibrillation had had some periods of rapid irregular heart rhythm and on one occasion series of ventricular extrasystoles had been recorded. One patient (case 12) had several periods of atrial flutter. One patient (case 16) described short series which might be extrasystoles and which she felt several times every week. Four of the 35 patients with sinus rhythm had thus had repeated periods of distressing symptoms caused by irregular or rapid heart rhythm. Two patients (cases 20, 51) were considered to have had pulmonary emboli. Neither of these two patients had had emboli before operation. No patients had arterial emboli postoperatively.

Other diseases than heart disease in the material are seen in table B in appendix.

Clinical findings

Signs of congestive heart failure were found only in one patient (case 62) as described in detail above. One patient (case 12) had dyspnea at rest.

In the following chapters comparisons between pre- and postoperative investigations will be reported. Absolute postoperative values will be commented on only in exceptional cases.

The postoperative classification of the 60 patients according to N.Y.H.A. compared with the preoperative classification was: 14 patients remained in the same groups, 40 patients were classified in lower groups, four patients (cases 12, 20, 57, 59) in higher groups, and two patients (cases 62, 72) died during the postoperative hospital treatment. Six of the 40 patients with less symptoms postoperatively were transferred from group III to group I, 3 patients from group IV to group II, and one patient (case 53) from group IV to group I (Table 26). The

high fever of irregular type for several months. It was not possible to get positive blood cultures in the last months before her death. The autopsy showed a mitral ostium that admitted more than one finger and lesions on the mitral valve which agreed with bacterial endocarditis.

The fourth death (case 62) was a female, 47 years of age at operation and with atrial fibrillation for six months before operation, who had combined stenosis and insufficiency in both aortic and mitral valves. By means of angiocardiography the mitral insufficiency was estimated to be mild and the aortic insufficiency of moderate degree. She had a diastolic mean pressure gradient over the mitral valve of 8 mm Hg and a systolic pressure gradient over the aortic valve of 30—45 mm Hg at the preoperative investigation. A diastolic mean pressure gradient of 8 mm Hg over the tricuspid valves was also found at operation the mitral valve admitted one finger and a regurgitation of moderate degree was palpated. The valves were split with a dilator and the aortic valve was also slightly dilated with the instrument. During the next year the patient suffered from progessive exertional dyspnea and she had increasing signs of congestive heart failure The patient died one year and three months after operation after several attacks of pulmonary edema. The autopsy showed a marked hypertrophy of the left ventricle, a mitral ostium which scarcely admitted the fifth finger and an aortic ostium that admitted a pencil The valves were very thick and immobile. No stenosis of the tricuspid valves was found.

Thus of 70 patients one died during the postoperative course, giving a *hospital mortality* of 1—2 % and 3 died during the follow-up period, corresponding to a late mortality during this period of 4—5 %

Table 27

Chang in classification acc rding to the criteria of New York Heart association from pr to post perative examination in different groups.

	Dead	Not Improved (NI)		Improved (I)	Total number
		Worse	Unchanged		
♂	—	2	5	11	18
♀	2	2	9	29	42
45 years at op or more	1	3	6	11	21
Less than 45 years at op	1	1	8	29	39
Sinus rhythm at operation	1	2	9	27	39
Atrial fibrillation at operation	1	2	5	13	21
Sinus rhythm at post op. examination	1	1	7	26	35
Atrial fibrillation postop. examination	1	3	7	14	25
G oup A	1	1	7	23	32
G oup B	1	3	7	17	28
Group 1	1	1	8	32	42
Group 2	1	3	5	8	17
Group S	2	3	9	21	35
Group L	—	1	4	19	24
Slight degree of or no calcification or fibrosis	—	2	4	16	22
More marked degree of calcification and or fibrosis	—	2	8	19	29

nd time were grouped as improved post operatively (group I) Thus the ppearance of a third sound occurred in patients with good functional results of the operation. In three patients (cases 51 54, 57) a third sound udible before operation was not re corded after operation.

Palm nary jection sound

Four patients (cases 2, 8 28 47) developed pulmonary ejection sound after the preoperative examination and one patient (case 15) lost such sound. Three of the first four patients were grouped as improved (group I)

Table 26
Groups according to the New York Heart Association

Number of patients in preoperative groups		Number of patients in postoperative groups				
		I	II	III	IV	Late Death
I	6	6	—	—	—	
II	19	9	7	3	—	
III	29	6	19	1	1	2
IV	6	1	3	2	—	
Total	60	22	29	6	1	2

40 patients with less symptoms postoperatively were classed in group I (Improved) and the other 20 patients in group NI (Not improved)

Comments

These pre postoperative changes in classification are related to sex, age heart rhythm and to the different groups of valvular anatomy in table 27

The incidence of patients grouped as improved (group I) and as not improved (NI) was not significantly different in the complementary groups Three of the four patients who had more symptoms from the heart after operation were above 44 years of age *These findings were consistent with* those of Stewart & Glenn (1959) These authors found furthermore the best results of *valvulotomy estimated as above according* to the classification of N Y H.A., in patients with tight mitral stenosis defined in about the same way as *in the present material* The same conclusions could not be confidently drawn from the present material, even though in the group with *increased* heart symptoms after operation most of the patients were classed in group 2 In agreement with the present results Stewart & Glenn *found satisfactory results approximately* as frequently in the group of pure mitral stenosis as in the groups of combined mitral valvular lesions and of mitral stenosis combined with aortic insufficiency of slight degree, but they observed more patients with bad postoperative results in the group of combined valvular lesions.

Stewart & Glenn also found the best postoperative results in patients in whom the split of the valves was judged at operation to be adequate, as well as in patients with no or only slight degree of calcification of the *mitral valves In the present material* there was no difference between the postoperative results for these groups.

Phonocardiographic findings

Post-preoperative differences (Table 28)

Third sound

A third sound appeared after operation in 10 patients, five in each of groups with a satisfactorily (S) and less satisfactorily (L) *split mitral valve Eight of these patients had* tight mitral stenosis at operation (group 1)

Opening snap

In 10 patients an opening snap had disappeared since the preoperative investigation. This finding could not be related to the tightness of the valve, the existence or not of calcification of the valves, the completeness of the split of the mitral valve, or to the functional postoperative status of the patients. In two patients (cases 18 44) an opening snap had appeared at the post operative investigation.

Pansystolic murmurs

A high-frequency pansystolic murmur with maximum over the apex had appeared since the preoperative examination in 15 patients and 14 of these patients had a satisfactory split of the mitral valve (group S) 10 had fibrous and/or calcification of the valves of moderate or marked degree. In four of them such changes were not palpated or were of slight degree in one patient there was no comment on the status of the valves in the operative record. Such pansystolic murmurs were approximately as frequent in patients grouped as improved (I) as in those grouped as not improved (NI). One patient (case 36) had postoperatively lost a pansystolic murmur with maximum over the lower part of the sternum indicating tricuspid insufficiency in another (case 55) such a murmur was recorded both pre and postoperatively

Diastolic murmur

Three patients (cases 18, 25 41) without apical diastolic murmurs recorded before operation had such murmur in the post operative recording In two patients (cases 16, 56) such a murmur had disappeared. The latter two patients were grouped both in S and I Five patients (cases 4, 11 14, 19 37) with an apical diastolic murmur of long duration before operation had post operatively only short murmur at the time normally occupied by the phase of rapid filling Four of these five patients had a tight mitral stenosis (group 1) before the valvulotomy and three were also grouped as improved (I) In four patients an early diastolic regurgitation murmur was recorded for the first time after operation.

Atriosystolic murmur

An atriosystolic murmur had disappeared at the postoperative examination in 15 patients, 10 of them in group S, and 11 in group I.

Time relations (Table 29)

There was no significant mean difference between the pre and postoperative duration of the heart cycle before that in which Q—1 and 2—OS intervals were measured.

Q—1 interval

The pre postoperative mean difference of the Q—1 interval in 59 patients, + 0.003 ± 0.003 sec, was not significant. Nor were there any significant pre-postoperative mean differences in the different groups.

2—OS interval

In the 42 patients with opening snap recorded both pre and postoperatively the mean value of the 2—OS interval in the postoperative recording was significantly higher, 0.008 ± 0.003 sec, than in the pre operative A corresponding post-preoperative significant mean increase was found also in the group of tight mitral stenosis (group 1) A probably significant mean increase was observed in groups B I and S (Definitions of groups in table 70)

A pre and postoperative mean difference between Q—1 and 2—OS intervals was cal

Table 28

Comparisons between pre and postoperative phonocardiograms Figures denote number of pat ents

Groups	Opening Snap		Ma ked shorten ing of diastolic murm r	D astolic apical murmur		Atrio-systolic murmur		Appear ance of early diastolic regurg. murmur	High frequency apical pansystolic murmur		Th rd sound	
	Appear ance	Disap-pearance		Appear ance	Disap-pearance	Appear ance	Disap-pearance		Appear ance	Disap-pearance	Appear ance	Disap-pearance
1	2	7	4	3	1		12	4	11		8	3
2	0	3	1		1		3		4		1	
Total	2	10	5	3	2	0	15	4	15	0	9	3
S	1	6	3	1	2		10	2	14		5	2
L	1	4	2				4	2	1		5	1
Total		10	5	3	2	0	14	4	15	0	10	3
I		6	3	3	2		11	2	9		9	1
NI	—	4	2				4	2	6		1	2
Total		10	5	3	2	0	15	4	15	0	10	3
Degree of calcification I	0	3	2	1			7	2	4		3	1
II	2	4	3	1	2		7	1	10		5	2
Total	2	7	5	2	2	0	14	3	14	0	8	3

however regarded a third heart sound that appeared after operation as evidence of at least moderate mitral incompetency. He found that 60 % of cases with trivial degree of mitral incompetency before operation developed a third sound after valvulotomy and that these patients often did very well functionally after operation. Only 7.5 % of cases without incompetency before operation developed a third sound postoperatively in his study. The incidence of a third sound in cases without postoperative mitral insufficiency was not reported.

In all studies of the incidence of a third sound the definition of such a sound has a decisive significance for the conclusions. The definition of the sound in the present study agrees with that used by McKusick (1958) in his discussion of findings in mitral stenosis. A third sound disappearing postoperatively is regarded as uncommon (Dack et al., 1960). All the three patients who had lost a third sound in the postoperative recording in the present material had a palpable mitral regurgitation at operation, and in two of them it was noted in the operative record that the regurgitating flow was slightly increased after valvulotomy. A changed sound transmission may have caused the disappearance of the third sound in these patients.

In the material of Wood (1954) mitral valvulotomy resulted in the loss of an opening snap in 32 % of the cases. In the present material the corresponding figure was 20 %. There was no opening snap recorded at the postoperative examination in 18 of the 60 patients.

The lack of correlation between the functional result of the valvulotomy and an induced mitral insufficiency indicated by the appearance of a pansystolic apical murmur agrees well with the findings of Stewart & Glenn (1959). In 170 operated patients they observed good functional results in most of the cases with a slight or moderate mitral insufficiency produced at operation. The mean follow-up time was 16 months.

The functional result of the operation in patients who lost their presystolic murmur after operation was not as good as in the study of Wood (1954). Fifty-eight per cent of his patients lost their presystolic murmur after operation. In two-thirds the operative results were excellent and this figure increased to 90 % if cases with a serious degree of mitral incompetency caused by the operation were excluded.

Many studies have shown a fairly good correlation between Q—1 and 2—OS intervals, on the one hand, and the tightness of mitral stenosis and/or pressures in the left atrium on the other (Wells, 1954, Wolter et al., 1955, Wells, 1957). Changes in these intervals, in some studies corrected for variations in preceding cycle length, together with the value of the difference between these intervals according to Wells (1954) have been used to assess the results of valvulotomy. The pre-postoperative changes of the 2—OS interval, and in some degree of the Wells index, in the present material were consistent with the findings in those studies but the changes in Q—1 intervals were not. As all intervals in the present study were measured after heart cycles of almost equal length in each patient pre- and postoperatively, it was not necessary to use values corrected for cycle length for evaluating the direction of changes of the intervals after operation.

Electrocardiographic findings

Post-preoperative differences (Table 37)
P mean vector in the frontal plane

In 26 patients with sinus rhythm both pre and postoperatively the P mean vector in

Table 29

Comparisons between pre and postoperative time relations on phonocardiograms in groups with differences of some significance

	Groups		n	$\bar{x}$	$\bar{d}$ post preop mean value	$\varepsilon_{\bar{d}}$	P
2—OS intervals in seconds	All patients	pre	42	0.073	+0.008	0.003	0.001—0.010
		post		0.081			
	B	pre	16	0.074	+0.013	0.005	0.01—0.05
		post		0.087			
	1	pre	28	0.069	+0.012	0.004	0.001—0.010
		post		0.081			
	I	pre	28	0.069	+0.009	0.004	0.01—0.05
		post		0.078			
	S	pre	26	0.075	+0.009	0.004	0.01—0.05
		post		0.084			
Diffe ence between Q—1 and 2—OS intervals in seconds	All patients	pre	41	+0.007	—0.012	0.004	0.001—0.010
		post		—0.005			
	S	pre	26	+0.002	—0.012	0.006	0.01—0.05
		post		—0 010			
	L	pre	15	+0.014	—0.011	0.005	0 01—0.05
		post		+0.003			

culated in 41 patients according to Wells (1954) The mean decrease of this index postoperatively was significant in the entire material and probably significant in groups S and L. The postoperative mean value of the index was also lower in group S than in group L, and this difference between the groups was probably significant.

Comments

Six of the 10 patients who had a third heart sound after operation did not have any pansystolic murmur over the apex either before or after operation. Thus in these cases the third sound did not seem to be related to an increased diastolic flow caused by a mitral insufficiency Wood (1954)

Table 30 continued

	Groups			$\bar{x}$	$\bar{d}$	e_d	P
Net area CR — CR mm²	All patients	pre	58	+22.6	+ 7.4	2.3	0.001—0.010
		post		+30.0			
	B	pre	28	+23.4	+12.4	3.9	0.001—0.010
		post		+35.8			
	I	pre	40	+21.4	+ 8.5	2.9	0.001—0.010
		post		+29.9			
	S	pre	34	+25.5	+ 7.6	2.6	0.001—0.010
		post		+33.2			
	NI	pre	20	+26.3	+11.6	4.9	0.01—0.05
		post		+37.9			

in 11 patients, corresponding to a mean change of $-12.8 \pm 2.9°$ highly significant difference. A decrease in mean vector of some significance was also found in most of the groups.

In the 33 patients classed as improved (*group I*) the QRS mean vector had shifted to the left in 27 and to the right in four patients. The postoperative change in mean value of QRS mean vector ($-17.7 \pm 3.8°$) was more marked in this than in the other groups.

The corresponding mean change in group NI was not significant. In this group of 17 patients 8 had shift to the left and 6 shift to the right of the QRS mean vector.

Net area CR_7—CR_2

The mean value of the net area of CR_7—CR was significantly increased after operation in 58 patients. There was a significant corresponding increase also in groups I, S and B and a probably significant mean increase in group NI (Definitions of groups in *table 20*).

Of the 8 cases with signs of right ventricle hypertrophy before operation 5 had lost these signs, 2 had them still and one had got left bundle branch block (case 33). One patient had got signs of right ventricle hypertrophy since operation.

Comments

The postoperative mean value of P mean vector was, like the preoperative, within the normal range (Grant, 1957).

Soulié et al. (1952) observed a change of the electrical axis of the P wave from left to right in 13 of 31 cases after operation. In the present material the P mean vector was within normal limits before operation, so no great changes in direction were to be expected as a result of valvulotomy. Furthermore, in cases with high pressures in the pulmonary circulation and right ventricle

Table 30
ECG
Post preoperative differences of some significance in different groups

	Groups		n	$\bar{x}$	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
P *mean vector* in frontal plane degrees	All patients	pre	26	+43.3	+10.4	4.1	0.01—0.05
		post		+53.7			
	A	pre	17	+43.8	+14.7	6.3	0.01—0.05
		post		+58.5			
QRS vector in frontal plane, degrees	All patients	pre	51	+68.9	—12.8	2.9	<0.001
		post		+56.1			
	A	pre	28	+67.4	—11.3	3.6	0.001—0.010
		post		+56.1			
	B	pre	23	+70.8	—14.6	4.8	0.001—0.010
		post		+56.2			
	1	pre	33	+71.4	—14.9	3.9	<0.001
		post		+56.5			
	2	pre	17	+63.7	— 9.6	4.1	0.01—0.05
		post		+54.1			
	S	pre	19	+69.0	—11.3	3.3	0.001—0.010
		post		+57.8			
	L	pre	22	+68.8	—14.7	5.1	0.001—0.010
		post		+54.1			
	I	pre	33	+69.9	—17.7	3.8	<0.001
		post		+52.2			

the frontal plane had shifted to the right in 10 patients and was unchanged in 12 patients after operation, corresponding to a probably significant mean increase of $+ 10.4 \pm 4.1$. In the group of pure mitral stenosis (group A) the postoperative increase of the P mean vector was also probably significant. In the nine patients in group B the P mean vector was unchanged in five cases and shifted to the right in two cases.

QRS mean vector in the frontal plane

In 51 patients after operation there was a shift to the left of the QRS mean vector in the frontal plane in 34 and to the right

Table 30 contin ed

	Groups			-	$\bar{d}$	t_d	P
Net are CR — CR curve	All patients	pre	58	+22.6	+ 7.4	2.3	0.001—0.010
		post		+30.0			
	B	pr	28	+23.4	+12.4	3.9	0.001—0.010
		post		+35.8			
	1	pre	40	+21.4	+ 8.5	2.9	0.001—0.010
		post		+29.9			
	S	pre	34	+25.5	+ 7.6	2.6	0.001—0.010
		post		+33.2			
	NI	pre	20	+26.3	+11.6	4.9	0.01—0.05
		post		+37.9			

in 11 patients, corresponding to mean change of — 12.8 ± 2.9° a highly significant difference. A decrease in mean vecto of some significance was also found in most of the groups

In the 33 patients classed as improved (group I) the QRS mean vector had shifted to the left in 27 and to the right in four patients The postoperative change in mean value of QRS mean vector (— 17.7 ± 3.8°) was more marked in this than in the other groups.

The corresponding mean change in group NI was not significant In this group f 17 patients 8 had shift to the left and 6 a shift to the right of the QRS mean vector.

N t are CR_7—CR_2

The mean value of the net area of CR_7—CR_2 was significantly increased after operation in 58 patients. There was a *signi* ficant corresponding increase also in groups I S and B and a probably significant mean increase in group NI (Definitions of groups in table 20)

*Of the 8 cases with signs of right ven*tricle hypertrophy before operation 5 had lost these signs, 2 had them still and one had got left bundle branch block (case 53) One patient had got signs of right ventricle hypertrophy since operation.

Comments

The postoperative mean value of P mean vector was, like the preoperative, within the normal range (Grant, 1957)

Soulié et al. (1952) observed change of the electrical axis of the P wave from left to right in 13 of 31 cases after operation. In the present material the P mean vector was within normal limits before operation, so no great changes in direction were to be ex pected as result of valvulotomy Further more, in cases with high pressures in the pulmonary circulation and right ventricle

hypertrophy there is a hypertrophy of both right and left atrium and, as seen in cases with chronic cor pulmonale, a hypertrophy of the right atrium changes the P mean vector antero-inferiorly and to the right. Thus a decrease of the pressures in pulmonary vessels as a result of valvulotomy may change the P vector in different directions depending on the degree of hypertrophy of the two atria before and after the operation

The postoperative mean value of QRS mean vector was like the preoperative within the normal range (Grant 1957)

Biörck et al (1953) found a general tendency to a shift of the electrical axis of the QRS complex to the left after mitral valvulotomy in patients both with good and with poor results of operation. They believed the cause to be a changed postoperative anatomic position of the heart. In the present material, however the change of the QRS mean vector in the frontal plane was a good indicator of the clinical result of the valvulotomy judged from the mean change in group I A change of the QRS mean vector in the frontal plane to the left was found by Demerdash & Goodwin (1963) in their whole group of 25 patients after valvulotomy This change was attributed to regression of right ventricular hypertrophy Their follow-up period lasted one year In cases with restenosis of the mitral valves the QRS mean vector was shown to shift to the right again in the same study

In the attempt to get a cardiographic criterion for the activity of the left ventricle Demerdash & Goodwin (1963) used the voltages $RV_5 + SV_1$ Changes in this index can be assumed to follow changes in QRS mean vector in the horizontal plane rather closely In their study they observed an increase of this index after valvulotomy in the group of tight mitral stenosis with little or no mitral incompetency They found a greater increase in a group with moderate or severe mitral incompetency after valvulotomy

The probably significant postoperative mean increase in net area $CR_7 - CR_2$ in the NI group in the present material in contrast to the insignificant mean change in group I may also be related to a higher frequency of postoperative mitral insufficiency in the first group Insufficiency was palpated at operation after valvulotomy in 15 of 18 patients in that group but only in 20 of 37 patients in group I.

More important than the existence of postoperative mitral insufficiency is, of course, the degree of the insufficiency the evaluation of which is difficult by the methods used in the present study The higher absolute mean value of the net area both pre and postoperatively in group NI than in group I indicated that the QRS mean vector was directed more posteriorly in that group The significant changes after operation in groups I and S may accord with an increase of the electrical forces of the left ventricle during depolarisation in that group, but also with a decrease of the same forces of the right ventricle A change in the net area of $CR_7 - CR_2$ after operation was thus less related to clinical results of operation than the change in QRS mean vector in the frontal plane

A change of QRS mean vector superiorly and posteriorly during a follow up period may be an effect of the increased age of the patients. In the present material the average follow up period was 34.8 months so that the effect of age may be disregarded according to the reported relations between QRS vectors and age (Lepeschkin 1951 Simonsson, 1961)

Values of exercise tests

Post-preoperative differences

Maximal work intensity in sitting position

In all the 58 patients who could perform this test both pre- and postoperatively there was a probably significant mean increase of + 58 ± 22 kpm/min after operation (table 31). Seven patients experienced a change of heart rhythm from sinus rhythm to atrial fibrillation, and two from atrial fibrillation to sinus rhythm after operation. If these patients were eliminated, the mean difference was about the same (+ 55 kpm/min) and the degree of significance unchanged.

Three of the seven patients who changed from sinus rhythm to atrial fibrillation had increased values of maximal work intensity the post-preoperative differences varying from 150 to 400 kpm/min. The other four patients had lower values than before operation, the differences ranging from 35—135 kpm/min. Of the two patients who had changed from atrial fibrillation to sinus rhythm after operation, one had a higher maximal work intensity the other a lower the differences being 250 and 100 kpm/min respectively.

Seven of the patients in the whole group had an increase of 300 kpm/min or more after operation. If 500 and 800 kpm/min, respectively is considered to be the lower limit of normal variation of maximal work intensity in females and males, there were 7 females and 4 males within the normal range after operation. Three of these patients were within the same range before operation.

In the 32 patients with sinus rhythm both pre and postoperatively there was a significant mean increase in maximal work intensity by 86 ± 30 kpm/min in the 17 patients with atrial fibrillation both pre and postoperatively there was no significant mean difference.

In the groups of pure mitral stenosis (group A) of tight mitral stenosis (group 1) of adequately split mitral valves (group S) and of improved patients (group I) there was a postoperative increase in maximal work intensity of some significance ($P<0.05$) between 52 and 116 kmp/min. In the complementary groups there were no significant changes. The greatest increase was found in group I, 116 ± 22 kpm/min.

In the 16 patients who had a tight mitral stenosis before operation and also an adequate split of the mitral valves at operation, there was a significant increase of the mean value by 147 kpm/min after operation.

In group I only six patients had lower values of maximal work intensity after operation, and in group NI only four patients had higher.

Maximal work intensity in supine position

The mean increase of maximal work intensity in supine position after operation was in all 51 patients who performed this test both pre and postoperatively 122 ± 24 kpm/min, a highly significant difference and the increase was as significant after elimination of the patients who had changed their rhythm after operation.

Post-preoperative differences of some significance were observed in the same groups in supine as in sitting position, but the mean increase in these groups was greater and of higher degree of significance. Thus in the group of 20 patients with sinus rhythm both pre and postoperatively the postoperative mean increase was + 154 ± 37 kmp/min, which was highly significant, while in the group of patients with atrial fibrillation pre and postoperatively no such significance was found.

Table 31

Bicycle ergometer test. Post-preoperative mean-differences. Maximal extrapolated work intensity in kpm/min

		Sitting position					Supine position				
		n	$\bar{x}$	$\bar{d}$	$s_{\bar{d}}$	P	n	$\bar{x}$	$\bar{d}$	$s_{\bar{d}}$	P
All patients	pre	58	383	+ 58	22	0.01—0.05	51	242	+122	24	<0.001
	post		441					364			
All patients with the same heart rhythm pre and postop.	pre	49	386	+ 55	24	0.01—0.05	42	246	+123	27	<0.001
	post		441					369			
All patients with sinus rhythm both pre and postop	pre	32	425	+ 86	30	0.001—0.010	29	269	+154	37	<0.001
	post		511					423			
Group A	pre	31	388	+ 52	27	0.05—0.10	29	242	+132	26	<0.001
	post		440					374			
Group A [1]	pre	26	386	+ 61	27	0.01—0.05	24	238	+153	27	<0.001
	post		447					391			
Group 1 [1]	pre	34	332	+ 92	24	<0.001	37	208	+153	25	<0.001
	post		424					365			
Group 3 [1]	pre	29	387	+ 78	33	0.01—0.05	22	235	+175	35	<0.001
	post		465					410			
Group I	pre	38	347	+116	22	<0.001	37	218	+149	24	<0.001
	post		463					367			

1 Patient with different heart rhythm pre- and postoperatively excluded.

Work intensity related to pulse difference $\frac{W_6}{\Delta f}$

Sitting positio

In all the 29 patients with sinus rhythm pre- and postoperatively there was after operation a highly significantly larger mean difference in $\frac{W_6}{\Delta f}$ of 1.86 ± 0.47 kpm/min/pulse beat (table 32) The table shows that in the patients with sinus rhythm there was in all groups a mean increase of some significance ($P < 0.05$) and the differences varied between 1.24 and 2.11 kpm/min/pulse beat. The highest degree of signif cance ($P<0.001$) was found in the improved patients (group I)

$\frac{W_6}{\Delta f}$ *supine p sition*

In supine position in the whole group of 24 patients with sinus rhythm both pre and postoperatively there was a highly significant mean increase of $\frac{W_6}{\Delta f}$ 1.98 ± 0.30 kpm/min/pulse beat after operation.

In the patients with sinus rhythm in the different groups there was, as in sitting position, postoperative mean increase in $\frac{W_6}{\Delta f}$ of some significance ($P<0.05$) in all groups. The most significant increase ($P<0.001$) was in groups S, 1 and I.

T summarize the results of all exercise tests the most significant post-preoperative increases in maximal work intensity and in $\frac{W_6}{\Delta f}$ ere found in the large groups of improved patients (group I) and of patients with tight mitral stenoses (group 1) In the smaller groups L and NI there were no significant post preoperative differences in maximal work intensity and only low degrees of significance in $\frac{W_6}{\Delta f}$ except in group L In supine position.

Comments

Cardiac output and exercise performance have been shown to be improved after conversion to sinus rhythm in patients with mitral stenoses and atrial fibrillation (Gilbert et al. 1963) Of the two patients (cases 22, 23) in the present study with such a change of heart rhythm after operation only one had higher values of maximal work intensity after operation.

Seven patients had changed their heart rhythm in the opposite direction after operation. Three of them had higher maximal work intensities after operation in sitting position and four of them in supine position. One patient had an unchanged value in the supine position. Thus in supine position only two of these seven patients had lower values of maximal work intensity after operation.

In the whole material the exclusion or not of the patients who had changed their heart rhythm after operation did not change the degree of significance of the post preoperative mean differences in maximal work intensity either in sitting or supine position.

The hemodynamic effect of change of heart rhythm after operation might in some of the groups conceal or magnify the effect of the heart operation. In groups A, B, 1 2, S and L all patients who had changed their heart rhythm since operation were excluded in the statistical analysis. Between groups I and NI the number of patients who changed from sinus rhythm to atrial fibrillation were about equally divided. One of the two patients who changed from atrial fibrillation to sinus rhythm belonged to

6

Table 32

Bicycle ergometer test Post preoperative mean differences in $\frac{W_6}{\Delta f}$

		in sitting position					in supine position				
		n	$\bar{x}$	$\bar{d}$	$\varepsilon_{\bar{d}}$	P	n	$\bar{x}$	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
All pat ents with sinus hythm both pre- and postop	pre post	29	5.52 7.38	+1 86	0.47	<0.001	24	4.57 6.55	+1 98	0.30	<0.001
Group 1	pre post	21	5 00 7 11	+2.11	0.62	0.001—0 010	18	4 19 6 06	+1 87	0.30	<0.001
Group 2	pre post	7	6 83 8.27	+1 44	0.52	0 01—0.05	5	5 62 8 04	+2.42	1.07	0.01—0.05
G oup S	pre post	18	5 40 7.36	+1 96	0.54	0.001—0.010	13	4 91 7 23	+2.32	0 44	<0.001
Group L	pre post	11	5.72 7 41	+1.69	0.91	0.05—0.10	11	4 17 5.75	+1.58	0.39	0.001—0.010
Group I	pre post	22	5.08 7 14	+2.07	0.48	<0.001	20	4.31 6.31	+2.00	0.37	<0.001
Group NI	pre post	7	6.90 8 14	+1.24	0 41	0.01—0.05	4	5 88 7 80	+1 92	0 43	0.01—0 05

group 1. No changes in degree of significance occurred if these patients with changed heart rhythm after operation were excluded from these two groups in the statistical analysis. The nine patients who had changed their heart rhythm after operation were about equally divided also between the other 6 groups.

It may be assumed that physiological and other factors which differ before and after a heart operation may influence the test of maximal work intensity. The work intensity pulse difference ratio is less influenced by such factors. As the post-preoperative mean differences of maximal work intensity accorded well with those of $\frac{W_6}{\Delta f}$ both in the whole material and in the different groups, there is strong indication of the unimportance of such factors for the significance of post-preoperative differences in maximal work intensity.

As noted above the $\frac{W_6}{\Delta f}$ was calculated at heart rates during exercise which were almost equal pre and postoperatively. The heart rate at the preoperative examination of all 29 patients in whom $\frac{W_6}{\Delta f}$ was measured was 138.2 ± 3.0 beats/min and after operation 136.4 ± 2.8 beats/min.

Heart volume in supine position

Post-preoperative differences

No significant post preoperative differences in heart volume were found in patients with the same heart rhythm pre and postoperatively either in those with sinus rhythm or atrial fibrillation or in the different groups.

In six patients there was postoperative decrease of the heart volume of 20 % or more. All these six patients had a tight mitral stenosis (group 1) before operation.

In two patients (cases 26, 52) there was an increase of the heart volume after operation of 20 % or more (+ 125 and + 290 ml respectively). The first had sinus rhythm both before and after operation and a tight mitral stenosis (group 1) before operation, but the split of the mitral valve was inadequate (group L) no postoperative mitral insufficiency was palpated at operation. The other patient had atrial fibrillation both pre and postoperatively and a less tight mitral stenosis (group 2) before operation and the mitral valve was split to a width of two fingers. There was a well palpable mitral insufficiency both before and after the commissurotomy.

Comments

The correlation between functional results of commissurotomy and pre-post operative changes in heart volume varies in different studies. Jiderberg & Nilsson (1964) found a mean decrease of heart volume calculated from exposures in standing position, of 162 ml in 20 of their 33 patients in the preoperative group III according to N.Y.H.A. classification.

In the material of Stewart & Glenn (1959) there was no close correlation between the anterior-posterior heart size and the clinical result of operation. Thus in 13 of 28 of their patients improved by mitral valvulotomy they found an increase in anterior posterior heart size.

Total amount of hemoglobin

Post-preoperative differences

In the whole group of 53 patients in whom total hemoglobin (THb) was estimated both pre and postoperatively there was significant mean decrease of 0.7 ± 0.2 g THb per kg body weight after opera

Table 33

Total amount of hemoglobin (THb) and blood volume (BV) related to body weight (Bwt) Comparisons between pre and postoperative values in different groups Only differences of some degree of significance are included

	Groups		n	$\bar{x}$	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
	All patients	pre	53	9.6	— 0.7	0.2	0.001—0.010
		post		8 9			
	Females	pre	37	9 4	— 0.8	0.2	<0.001
		post		8.5			
THb	A		29	—	— 0.8	0.2	0.001—0.010
g/kg	1		39	—	— 0.5	0.2	0.01—0.05
	2		14	—	— 1.0	0.3	0.01—0.05
	S		28	—	— 0.7	0.3	0.01—0.05
	I		36	—	— 0.7	0.3	0.001—0 010
	All patients	pre	51	82.9	— 5 9	1.8	0.001—0.010
		post		77.0			
	Females	pre	37	82.9	— 7 2	2.0	0.001—0.010
		post		75.6			
BV	A		29	—	— 8.2	2.2	0.001—0.010
ml/kg			13	—	—11.3	3.5	0.001—0.010
	S		26	—	—10.5	2.5	<0.001
	I		35	—	— 6.5	3.0	0.001—0.010

tion (Table 33) A corresponding highly significant mean decrease of 0.8 ± 0 2 g/kg was found in the group of 37 women no significant difference was found in the group of 16 men.

In the different groups *there was a post* operative decrease of some significance (P<0 05) *in the groups of* pure (group A) tight (group 1) and less tight (group 2) mitral stenosis, and also in patients who had adequately split valves (group S) and in those who were improved (I) The mean decrease ranged from 0 7 to 1 0 g/kg and the most *significant increase (0 001<P<0 010) was found in groups A and I In the group of* not improved patients (group NI) and of those with less satisfactorily split valves there were no significant changes

Concentration of hemoglobin and blood volume

No post-preoperative difference of any significance was found in the mean hemoglobin concentration either in females or in males. The mean differences in these two groups were only 0.2 %

In the 51 patients in whom blood volume was estimated both pre and postoperatively there was significant mean decrease of 5.9 ± 1.8 ml/kg body weight after operation. The mean decrease in the female group, 7.2 ± 2.0 ml/kg, was significant but not that in the male. In groups A, 2 and I there were significant post-preoperative mean decreases between 6.5 and 11.3 ml/kg. The mean decrease of highest degree of significance (P<0.001) was in group S 10.5 ± 2.5 ml/kg.

Summarizing the results f the determinations of total hemoglobin and of blood volume, the postoperative compared to the preoperative mean values were lower in the females, in the groups of patients with pure mitral stenosis, in those with less tight stenosis and those with adequately split mitral valves and also in those who according to the N.Y.H.A. classification were "Improved" The postoperativ mean value of total hemoglobin was also lower in group 1

Comments

In the present material there was significant mean increase in body weight of 1.5 ± 0.6 kg after operation. Even if relations between blood volume and total hemoglobin on the one hand and body weight on the other were not constant in each individual, such small changes in body weight should not introduce any significant error in the post-preoperative differences either in total hemoglobin or in blood volume related to body weight.

Both total hemoglobin and blood volume are positively related to the degree of daily activity of an individual (Sjöstrand, 1953). In the present material, according to the histories of the patients and to the pre-post operative changes in N.Y.H.A. classification, most of the patients increased their daily activity after operation. This would result in an increase in blood volume instead of the decrease found in the present material. A possible cause of a decrease in blood volume would be the effect of digitalis and of diuretics. In the present material there were no significant differences in numbers of patients treated with these therapeutics before and after operation. Furthermore the greatest changes in total hemoglobin and blood volume were found in groups with the best clinical effect of commissurotomy and with few patients treated with these therapeutics

In study by Sjöstrand (1953) it was found that heart patients with stasis in the lesser circulation but not in the systemic had larger blood volumes than patients without such stasis. The finding in the present material of mean decrease in blood volume in patients with good clinical results of valvulotomy and also in patients with decreased pressures1) in the pulmonary circulation after operation, accorded well with that study

Likoff et al. (1955) found in group of 23 patients with rheumatic valvular disease selected for operation and with earlier decompensation a higher mean value of red cell volume than in group of healthy individuals, but the value was within normal limits.

I the present study the greatest increase both of total hemoglobin and of blood

1) Pressures in different groups commented on below

volume after operation + 3 5 g/kg and + 25 1 ml/kg respectively was found in patient 55 who at the pre-operative examination had signs of congestive heart failure and a large blood volume and had had edema in the legs since operation In spite of continuous treatment with digitalis and diuretics

Basal metabolic rate

Post preoperative differences

The post preoperative mean differences of the quotients between observed and predicted oxygen uptake at rest during basal conditions in all 51 patients in whom these estimations were made was + 0 02 ± 0 01 This mean difference was not significant.

Comments

An increased basal metabolic rate in patients with cardiac disease has been shown to be best correlated to degree of congestive heart failure and to respiratory rate (Lev & Hamburger 1925/26 Resnik & Friedman, 1935) In the present material there was only one patient after and none before operation who had a marked dyspnea at rest and only one both pre and postoperatively who had signs of congestive heart failure so that no post preoperative differences in basal metabolic rate of any significance were to be expected.

Spirometric values

Post-preoperative differences in deviations from predicted spirometric values

In the whole group of 57 patients highly significant post preoperative changes in spirometric values were observed only in functional residual capacity (FRC) and in the quotient between FRC and total lung capacity (TC) (table 34) The post preoperative mean difference in deviations from predicted values was −292 ± 77 ml in FRC and −5.8 ± 0 7 % in FRC/TC, corresponding to a postoperative increase of FRC and FRC/TC. Also in the different groups the most significant changes were found in FRC and FRC/TC. Thus FRC was highly significantly increased in groups A, 1 L and I and FRC/TC in all except the small group 2, in which the increase was probably significant. The mean increase in FRC ranged from 331 to 459 ml and in FRC/TC from 4.5 to 7 5 % The residual volume (RV) was probably significantly increased in groups A, L and I and vital capacity (VC) significantly decreased in groups 2 (330 ml) and NI (251 ml) The mean increase in RV ranged from 112—144 ml. The mean quotient RC/TC was probably significantly increased in groups A (2.4 %) and L (2 3 %)

Comments

Spirometric studies before and after commissurotomy in 11 patients studied by Dogliotto et al (1959) showed poor correlation between changes in spirometric values and clinical results of the operation. In the present study the most marked post preoperative changes were found in functional residual capacity the changes in residual volume were of less significance. Such an increase mainly in expiratory reserve volume may be a mechanical effect of the thoracotomy A further analysis was not possible by the methods used in the present study A decrease in vital capacity is a common finding in patients with increasing pulmonary congestion (Comroe et al., 1962) In the present study there was a significant decrease in vital capacity in the group of patients who were not improved according to the N Y.H.A. classification.

Table 34

Post p eoperative mean differences of some degree of signif cance in deviations from predicted spirometric al s n different groups

Lung volumes	Groups		$\bar{d}$	$s_{\bar{d}}$	P
FRC ml	All patients	57	−292	77	<0.001
	A	31	−425	75	<0.001
	1	40	−365	70	<0.001
	L	22	−459	69	<0.001
	I	38	−331	76	<0.001
RV ml	A	31	−144	61	0.01—0.05
	L	22	−145	64	0.01—0.05
	I	38	−112	51	0.01—0.05
VC ml	2	15	+330	110	0.001—0.010
	NI	18	+251	72	0.001—0.010
FRC/TC	All patients	56	−5.8	0.7	<0.001
	A	31	−6.2	1.0	<0.001
	B	25	−5.3	1.0	<0.001
	1	40	−6.2	0.8	<0.001
	2	15	−4.5	1.6	0.01—0.05
	S	34	−4.7	1.0	<0.001
	L	21	−7.5	1.0	<0.001
	I	38	−5.4	0.9	<0.001
	NI	18	−6.7	1.4	<0.001
RC/TC	A	31	−2.4	1.1	0.01—0.05
	L	21	−2.3	1.1	0.01—0.05

FRC = functional residual capacity
RV = residual volume
VC = vital capacity
TC = total lung capacity
All volumes in ml_{BTPS}

Heart catheterization

Post preoperative differences. Investiga tions at rest

Oxygen uptake

In the following sections dealing with post preoperative differences of A—V oxygen difference, stroke volume, pressure, right ventricle work, resistance in pulmonary and systemic circulation and specific ventila tion, comparisons between the different groups will be made as in earlier sections. To avoid too much repetition, only the most significant differences or those of special interest will be mentioned for the rest the reader will be referred to the tables. Some times the groups not mentioned will be referred to as the other groups" or by some equivalent expression. All post preoperative comparisons are intra individual as in earlier sections, except when covariance analysis is used Only the post preoperative compari sons of arterial oxygen saturation and oxygen uptake at rest and of peripheral vascular resistance index during exercise are made in all catheterized patients the other compa risons are made only in patients with the same heart rhythm pre and postoperatively

Oxygen uptake

The oxygen uptake at rest during the post operative heart catheterizations was generally less increased above the observed basal meta bolic rate than before operation. The mean deviation in percent of observed basal meta bolic rate was + 11 4 ± 1.2 % which was 9 5 % lower than the corresponding value in all the preoperative catheterizations This difference was significant.

Arterial oxygen saturation

The postoperative mean value of arterial oxygen saturation at rest in all the 52 pa tients catheterized both pre and postopera tively was not significantly different from the corresponding value before operation.

Arterio-venous oxygen difference

In all patients there was a significant post preoperative mean difference of + 4.8 ± 1 6 ml/l (table 36)

In the whole material 15 patients had a lower A—V oxygen difference postopera tively and 13 of them were in group I.

Figure 8 shows that almost all patients with atrial fibrillation and about half of those with sinus rhythm had an A—V oxygen difference above the upper limit of normal variation.

Th mean value of post preoperative differences was not significantly higher in

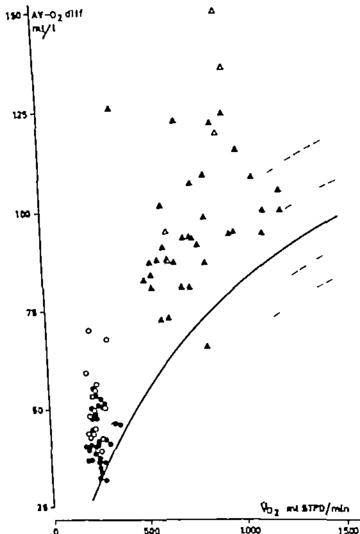
Table 35

Postoperative heart catheterization oxygen uptake ml$_{STPD}$ (x) and cardiac output l/min (y) at rest

Correlation and regression

Heart rhythm	n	$\bar{x}$ ml/min	$\bar{y}$ l/m		P	regression equatio	S y
Sinus rhythm	26	241	5.21	0.61	0.001—0.010	y = 0.019x + 0.65	0.79
Atrial fibrillation	17	236	3.85	0.650	0.001—0.010	y = 0.014 + 0.59	0.53

Fig. 8. A—V oxygen difference in relation to oxygen uptake ($\dot{V}_{O_2}$) at rest and during exercise after operation. Symbols and regression line as in figure 3



the atrial fibrillation than in the sinus rhythm group. In neither group was the mean value significantly different from zero.

There were significantly higher mean values of arterio-venous oxygen difference after than before operation in the groups A, 2, S and NI. The degree of significance was lower in most of the groups after exclusion of cases with atrial fibrillation. There were no differences of any significance in the larger groups of "improved" (group I) and of tight mitral stenosis (group 1).

Post operative

Cardiac output

There was a significant linear regression of cardiac output on oxygen uptake postoperatively both in the group of 26 patients

Table 36
Post preoperative differences at rest
Non significant differences are not included

	Groups	Rhythm	n	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
A—V oxygen difference ml/l	All patients	s+f	43	+4.76	1.64	0.001—0.010
	A	s+f	22	+6.03	1.98	0.001—0.010
	A	f	6	+10.17	3.52	0.01—0.05
	2	s+f	10	+10.35	2.49	0.001—0.010
	2	s	6	+8.85	2.06	0.001—0.010
	S	s+f	24	+5.33	1.75	0.001—0.010
	NI	s+f	14	+9.18	2.47	0.001—0.010
	NI	f	6	+11.53	4.10	0.01—0.05
Stroke volume ml	All patients	s	26	−5.8	2.7	0.01—0.05
	2	s	6	−14.8	2.7	0.001—0.010
Pulmonary arterial mean pressure mm/Hg	All patients	s+f	43	−12.6	2.2	<0.001
	All patients	s	26	−15.5	2.9	<0.001
	All patients	f	17	−8.2	3.1	0.01—0.05
	A	+f	22	−15.7	3.2	<0.001
	A	s	16	−17.6	3.7	<0.001
		s+f	21	−9.4	2.7	0.001—0.010
	B		10	−12.1	4.5	0.01—0.05
	1	s+f	32	−15.9	2.5	<0.001
	1		19	−18.6	3.5	<0.001
	1	f	13	−11.8	3.1	0.001—0.010
	2	s	6	−8.2	2.9	0.01—0.05
	S	s+f	24	−11.7	3.4	0.001—0.010
	S	s	15	−17.3	4.3	0.001—0.010
	L	s+f	19	−13.8	2.5	<0.001
	L		11	−13.1	3.4	0.001—0.010
	L	f	8	−14.8	3.8	0.001—0.010
	I	s+f	29	−17.2	2.5	<0.001
	I		18	−20.0	3.4	<0.001
	I	f	11	−12.5	3.5	0.001—0.010
Pulmonary wedge mean pressure mm/Hg	All patients	s+f	41	−8.4	1.4	<0.001
	All patients		25	−10.1	1.5	<0.001
	All patients	f	16	−5.6	2.5	0.01—0.05
	A	+f	22	−11.1	1.5	<0.001
	A	s	16	−11.6	1.7	<0.001
	A	f	6	−9.8	3.3	0.01—0.05

Table 36 continued

	Groups	Rhythm		$\bar{d}$	e_d	P
Pulmonary wedge mean pressure mm/Hg	B	+f	19	− 5.2	2.2	0.01—0.05
	B		9	− 7.6	2.8	0.01—0.05
	1	+f	30	−10.5	1.5	<0.001
	1		18	−12.1	1.8	<0.001
	1	f	12	− 8.2	2.5	0.001—0.010
	2		6	− 7.8	1.7	0.001—0.010
	S	+f	23	− 7.2	1.9	<0.001
	S		14	−10.5	1.9	<0.001
	L	+f	18	− 9.8	2.0	<0.001
	L		11	− 9.6	2.6	0.001—0.010
	L	f	7	−10.1	3.4	0.01—0.05
	I	+f	26	−11.2	1.5	<0.001
	I		17	−12.8	1.6	<0.001
	I	f	9	− 8.2	3.0	0.01—0.05
Right ventricle end diastolic pressure mm/Hg	1	f	13	− 2.8	1.2	0.01—0.05
	L	f	8	− 3.9	1.5	0.01—0.05
Right ventricle work against pressure kpm min.	All patients	s+f	43	− 1.1	0.2	<0.001
	All patients		26	− 1.4	0.2	<0.001
	All patients	f	17	− 0.6	0.2	<0.001
	A	+f	22	− 1.4	0.2	<0.001
	A		16	− 1.6	0.3	<0.001
	A	f	6	− 0.8	0.3	0.01—0.05
	B	+f	21	− 0.8	0.2	<0.001
	B		10	− 1.1	0.3	0.01—0.05
	B	f	11	− 0.6	0.2	0.01—0.05
	1	+f	32	− 1.2	0.2	<0.001
	1		19	− 1.5	0.3	<0.001
	1	f	13	− 0.7	0.2	<0.001
	2	+f	10	− 0.9	0.3	0.001—0.010
	2		6	− 1.2	0.4	0.01—0.05
	S	+f	4	− 1.1	0.2	<0.001
	S		15	− 0.4	0.2	0.01—0.05
	S	f	9	− 1.1	0.2	<0.001
	L	+f	19	− 1.2	0.3	<0.001
	L		11	− 0.9	0.2	0.001—0.010
	L	f	8	− 1.5	0.3	<0.001
	I	+f	29	− 1.3	0.2	<0.001
	I		18	− 1.6	0.3	<0.001
	I	f	11	− 0.7	0.2	0.001—0.010
	NI	+f	14	− 0.8	0.2	0.001—0.010
	NI		8	− 0.9	0.3	0.01—0.05

Table 36 continued

	Groups	Rhythm	n	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
Systemic artery mean pressure mm/Hg	I	s+f	29	+4.2	1.8	0.01—0.05
Pulmonary vascular resistance "units"	All patients	s	25	—0.9	0.4	0.01—0.05
	1	s+f	30	—1.2	0.5	0.01—0.05
	1	s	18	—1.3	0.5	0.01—0.05
	I	s+f	27	—1.5	0.5	0.001—0.010
	I	s	17	—1.4	0.5	0.01—0.05
Systemic vascular resistance index "units"	All patients	s+f	42	+3.3	0.8	<0.001
	All patients	s	25	+3.0	0.8	<0.001
	A	s+f	22	+3.2	0.9	0.001—0.010
	A	s	16	+3.0	1.1	0.01—0.05
	B	s+f	20	+3.4	1.5	0.01—0.05
	B		9	+3.0	0.9	0.001—0.010
	1	s+f	31	+2.9	1.1	0.01—0.05
	1	s	18	+[illegible].7	1.0	0.01—0.05
	2	s+f	10	+4.9	1.1	0.001—0.010
	2	s	6	+4.5	1.1	0.01—0.05
	S	s+f	23	+2.2	0.9	0.01—0.05
	S	s	15	+2.5	1.0	0.01—0.05
	L	s+f	17	+3.8	1.0	0.001—0.010
	L	s	10	+3.9	1.2	0.001—0.010
	I	s+f	28	+3.4	1.4	0.001—0.010
	I		17	+3.3	0.9	0.001—0.010
	NI	+f	14	+2.8	1.2	0.01—0.05
Specific ventilation ml_{BTPS} ml_{STPD}	All patients	+f	43	—2.7	1.1	0.01—0.05
	All patients		26	—5.1	1.9	0.01—0.05
	A	+f	22	—3.0	1.4	0.01—0.05
	A		16	—4.0	1.8	0.01—0.05
	1	+f	32		1.6	0.01—0.05
	1		19	—6.3	2.1	0.001—0.010
	S	s+f	24	—3.8	1.8	0.01—0.05
	S		15	—6.5	2.5	0.01—0.05
	I	s+f	29	—4.0	1.6	0.01—0.05
	I		18	—6.9	2.1	0.001—0.[illegible]10

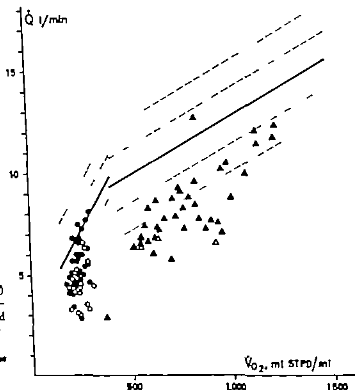


Fig. 9 Cardiac output ($\dot{Q}$) in relation to oxygen uptake ($\dot{V}_{O_2}$) at rest and during exercise after operation
Symbols and regression lines as in figure 4

with sinus rhythm and in the group of 17 patients with atrial fibrillation (table 35). The highly significant difference between adjusted means of cardiac output in the two groups was 1.28 l/min with the higher value in the sinus group. Figure 9 shows that almost all cases with atrial fibrillation but less than half of those with sinus rhythm had resting cardiac output below the normal range.

Cardiac output
Post-preoperative difference

The post-preoperative, non-significant difference in cardiac output between adjusted means in the 26 patients with sinus rhythm was −0.8 l/min. The post-preoperative non-significant difference in patients with atrial fibrillation was −0.6 l/min at an oxygen uptake of 238 ml/min.

The post-preoperative difference between adjusted means of cardiac output was largest in group S in the patients with sinus rhythm, −0.6 l/min, but was not significant.

Nor were there any significant post-preoperative differences between adjusted means of cardiac output in the other groups or subgroups, but in all groups the postoperative adjusted mean of cardiac output was lower then the preoperative.

Stroke volume in patients with sinus rhythm both pre- and postoperatively

In all 26 patients with sinus rhythm there was a probably significant mean decrease of the resting stroke volume after operation of

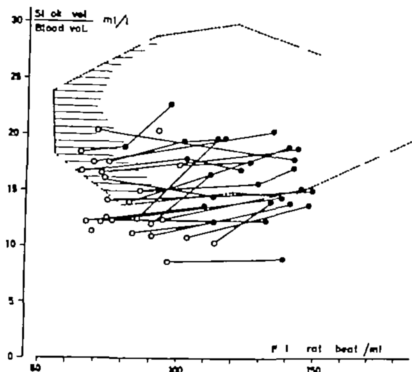


Fig. 10. Quotient between stroke volume and blood volume in relation to pulse frequency at rest (unfilled circles) and during exercise (filled circles) in patients with sinus rhythm after operation. Dotted line indicates normal range. Hatched area normal range at rest.

5 8 ± 2.7 ml. The heart rate during sampling for cardiac output was lower at the post operative investigation in 16 unchanged in 6 *and increased in 4 patients In those in* group 2 there was a significant decrease in stroke volume after operation of 14 8 ± 2 7 ml No significant post preoperative differences in stroke volume were found in any other group

The relation of stroke volume to blood volume was not significantly changed either in the whole material or in the different groups (fig 10)

Pulmonary artery mean pressure

In all 43 patients there was a highly significant mean decrease of the pulmonary artery mean pressure of 12.6 ± 2 2 mm Hg after operation. In the patients with sinus rhythm the mean decrease was 15 5 ± 2 9 mm Hg and of the same degree of significance, in the patients with atrial fibrillation 8 2 ± 3 1 mm Hg, a probably significant difference The mean difference in the first group was however not significantly greater than in the other

Of all patients with sinus rhythm only one (case 51) had an increased pulmonary artery mean pressure after operation, and of patients with atrial fibrillation only four (cases 19 21 57 59) had such an increase. All patients with increased pulmonary artery mean pressure belonged to group B except case 19 Thus they had combined valvular lesions before operation. All of them except case 51 belonged also to group S so they had an adequately split valve after operation If 20 mm Hg is regarded as the upper limit of normal variation for pulmonary artery mean pressure in these age groups 31 patients had reached this range after operation. Twenty of them had a mean pressure of 15 mm Hg or less In all groups except NI there was a postoperative mean decrease of some significance, varying between 8 and 20 mm Hg The latter value was

found in the patients with sinus rhythm in group I. Thus the most marked decrease in pulmonary artery mean pressure was in the group of clinically improved patients (group I) and the only group without significant mean change of the same pressure was the clinically not improved (group NI)

Pulmonary wedge mean pressure

In the whole group of 41 patients there was a highly significant mean decrease in pulmonary wedge mean pressure of 8.4 ± 1.4 mm Hg, and also in the 25 patients with sinus rhythm, 10.1 ± 1.5 mm Hg. In the 16 patients with atrial fibrillation the mean decrease, 5.6 ± 2.5 mm Hg, was probably significant.

Of all patients with sinus rhythm only two (cases 48, 51) had an increased pulmonary wedge mean pressure after operation. Of the patients with atrial fibrillation there were 5 patients with such an increase. Of the 7 patients with higher postoperative wedge mean pressure 5 belonged to group B and 5 also to group S.

In 20 of the patients the mean pressure had decreased 10 mm Hg or more after operation and in 9 patients the decrease was 15 mm Hg or more.

If 15 mm Hg is set as the upper normal limit for pulmonary arterial wedge mean pressure in these age groups, there were 37 patients within the normal range after operation. Nineteen of them had mean pressure of 10 mm Hg or less.

In agreement with the changes in pulmonary artery mean pressures there was a postoperative mean decrease of some significance in all groups except NI, the greatest mean decrease, 12.8 ± 1.6 mm Hg, being in the patients with sinus rhythm in group I

End-diastolic pressure in right ventricle

There were no post preoperative differences of end-diastolic pressure in the right ventricle of any significance either in the material as a whole or in the patients with sinus rhythm or with atrial fibrillation. In the different groups it was only in patients with atrial fibrillation in group I and in group L that a mean decrease of probable significance was found, 2.8 ± 1.2 mm Hg and 3.9 ± 1.5 mm Hg respectively

Right ventricle work against pressure

In all 45 patients there was mean decrease of right ventricle work against pressure of 1.1 ± 0.2 kpm/min, the corresponding mean decrease in the patients with sinus rhythm being 1.4 ± 0.2 and in the patients with atrial fibrillation 0.6 ± 0.2 kpm/min all these differences were highly significant. Of all patients with sinus rhythm there was only one (case 51) who had an increase in right ventricle work against pressure after operation, and of the patients with atrial fibrillation five (cases 5, 21, 42, 52, 59) Four of these six patients belonged to group B In all groups there was a mean decrease in right ventricle work against pressure of some significance, ranging from 0.4 to 1.6 kpm/min. The latter, highly significant difference, was found in groups A and L

Mean pressure in systemic artery

There were no significant post-preoperative mean differences in mean pressure in the systemic artery either in the whole material or in the patients with sinus rhythm or with atrial fibrillation. Fig. 11 shows the individual postoperative mean pressures related to cardiac output.

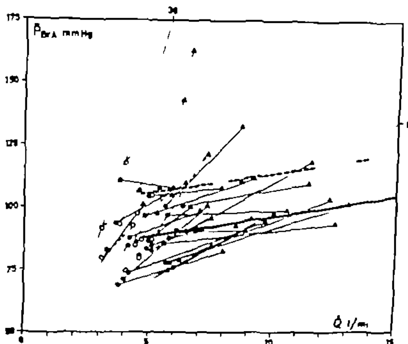


Fig. 11 Mean pressure in the systemic artery ($\bar{P}_{BrA}$) in relation to cardiac output ($\dot{Q}$) at rest and during exercise after operation. Individual symbols as in figure 4. Regression line (heavy line) + 2 S_y (broken heavy line) in healthy individuals.
The thin lines are no-resistance index lines.

In all patients in group I there was a probably significant mean increase of 4.2 ± 1 8 *mm* Hg *in the mean pressure of the* systemic artery in the other groups there were no significant post preoperative mean differences

Pulmonary vascular resistance

There was no significant post preoperative mean difference in pulmonary resistance either in the material as a whole or in the patients with atrial fibrillation Only in the patients with sinus rhythm was there a probably significant mean decrease of 71 2 ± 31 2 dynes sec cm^{-5} In six cases a decrease of *200 dynes sec cm^{-5} or more was ob*served. If 120 dynes sec cm^{-5} corresponding to an isoresistance index line between 1 and 2 in figure 12, is considered to be the limit of upper normal variation in these age groups, 26 patients had values above this limit (fig 12) compared to 30 before operation. A decrease in pulmonary resistance of some significance was found in groups 1 (96 ± 40 dynes sec cm^{-5}) and I (120 ± 40 dynes sec cm^{-5})

Peripheral vascular resistance

There was a higly significant increase in the mean value of peripheral resistance in the whole group of patients and in the patients with sinus rhythm, 266.4 ± 66.4 and 243 2 ± 60 0 dynes sec cm^{-5} respectively In the patients with atrial fibrillation there was no such significant difference. The values of mean increase in the two rhythm groups were however, not significantly different.

The individual relations between systemic mean pressure and cardiac output are seen *in figure 11 together with regression* and upper limit of normal variation for these relations. The figure shows that the resistance index values of the cases with *atrial* fibrillation were higher than in cases with sinus rhythm, in the same way as before operation. If 1800 dynes sec cm^{-5} is considered to be the upper normal limit of resistance index for the range of flows in this

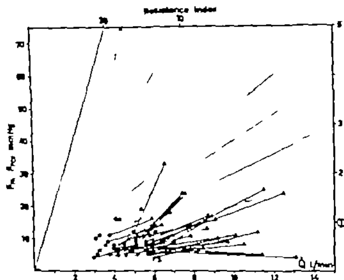


Fig. 12. The relation between pressure drop over peripheral pulmonary vessels ($\bar{P}_P$ — $\bar{P}_{PCV}$) and cardiac output ($\dot{Q}$) after operation. Individual symbols as in figure 4. The broken thin lines are isoresistance index lines. Upper limit of normal variation in healthy individuals reaches slightly above isoresistance index 1 (thin broken line).

material 15 patients had a resistance index value outside this limit compared to 6 before operation.

There was a mean increase in peripheral vascular resistance of some significance in all groups ranging between 176 and 392 dynes sec cm^{-5}. The lowest value was observed in group S and the highest in group 2.

Specific ventilation

In the whole material and also in all patients with sinus rhythm there was a probably significant decrease of the mean value of specific ventilation in ml_{BTPS}/ml_{STPD} postoperatively of 2.7 ± 1.1 and 3.1 ± 1.9 respectively.

If specific ventilation of 34 is considered as the upper limit of normal variation, 14 of the whole group had values above that limit compared with 25 before operation. A postoperative mean decrease of some significance ($P<0.05$) was found in groups A, 1, S and I. The mean decrease varied between 3.0 and 6.9. The latter value was found in group I.

Investigations during exercise

Arterial oxygen saturation

No significant pre-postoperative difference in arterial oxygen saturation was found in 30 patients who worked at the same or almost the same load pre- and postoperatively.

Respiratory quotient

The postoperative mean value of the respiratory quotient was not significantly different from the preoperative in 27 patients exercising at equal loads pre- and postoperatively.

All investigations of flows and pressures during exercise were made on 19 patients who had the same heart rhythm and worked at the same loads pre- and postoperatively. Only peripheral vascular resistance values were calculated also in patients with different heart rhythm pre- and postoperatively.

Arterio-venous oxygen difference

The post-preoperative mean difference of A—V oxygen difference was non-significant, −1.1 ± 2.4 ml/l. In group S the corre-

Table 37

Data from heart catheterization during exercise at equal loads pre and postoperatively Post preoperative differences The same heart rhythm pre and postoperatively except in part of table with resistance indices

	Groups	Rhythm	n	$\bar{d}$	$\varepsilon_{\bar{d}}$	P
A—V oxygen difference ml/L	All patients	s+f	19	— 1 1	2.4	>0.50
	S	s+f	13	— 5.5	2.1	0.02—0.05
	L	s+f	6	+ 8.5	4.3	>0.50
Cardiac output l/min.	All patients	s+f	19	+ 0.3	0.3	0.20—0.30
	S	s+f	13	+ 0.7	0.3	0.05—0.10
	L	s+f	6	— 0.6	0.4	0.10—0.20
Relative change in Stroke volume, %	All patients	s	15	+25 9	3 4	<0.001
Pulmonary artery mean pressure mm/Hg	All patients	s+f	19	—14.8	3.8	<0.001
	S	s+f	13	—19.6	3 9	<0.001
	L	s+f	6	— 4 1	6.9	>0.5
Pulmonary arterial wedge mean pressure mm/Hg	All patients	s+f	15	—10.8	3 9	0.01—0.05
	S	s+f	10	—14.2	5.2	0.01—0.05
	L	s+f	5	— 4.0	4.5	>0.5
Mean pressure in systemic artery mm/Hg	All patients	s+f	18	+ 4 4	3 0	>0.5
Peripheral vascular resistance index	All patients		25	+ 0.57	0.66	>0.2
	S		16	— 0.23	0.90	>0.5
	L		9	+ 1 99	0.69	0.01—0.05

sponding difference was probably significant —5 5 ± 2.1 ml/l (Table 37)

In group L the postoperative mean value was larger than the preoperative, 8.5 ± 4.3 ml/l, but the difference was not significant.

The difference between the groups S and L in post preoperative changes was significant.

Individual postoperative values are plotted in fig. 8

Cardiac output

The post preoperative mean difference of cardiac output of all patients during exercise at equal loads, + 0 5 ± 0 5 l/min, was not significant, nor was the corresponding value in group S, + 0 7 ± 0.3 l/min, or in group L, − 0.6 ± 0 4 l/min. There was a probably significant difference in the mean values between the two latter groups. Figure 9 shows that all cases with atrial fibrillation and most of the cases with sinus rhythm had values below the lower limit of normal variation.

Stroke volume

Of the 15 patients who had sinus rhythm pre and postoperatively there were 13 before operation who had lower values of stroke volume during work than at rest, corresponding to a mean decrease in stroke volume of 9 3 %. After operation there were 14 patients with higher values of stroke volume during work than at rest, corresponding to mean increase of 16.6 %. This post pre operative mean difference between the relative changes in stroke volume was 25.9 % which was highly significant.

Pulmonary artery mean pressure

The pulmonary artery mean pressure in the 19 patients during exercise was highly significantly lower after operation, the mean difference being 14.8 ± 3.8 mm Hg. The corresponding difference was even larger in group S, 19.6 ± 3 9 mm Hg, also highly significant in the six patients in group L there was a non-significant mean decrease of 4.1 ± 6.9 mm Hg. The difference between the mean values in these two groups was probably significant. Of all the 19 patients only three had higher mean pressure after operation (cases 4, 8, 23).

Arterial pulmonary wedge mean pressure

In 15 patients the mean value of pulmonary arterial wedge mean pressure was probably significantly lower, 10.8 ± 3.9 mm Hg, after operation, the difference being 14.2 ± 5.2 mm Hg in group S, probably significant. In the five patients in group L the postoperative mean value was only 4.0 mm Hg below the preoperative, not significant difference.

Only 4 of all the 15 patients had a higher mean pressure after operation (cases 6, 40 30, 51)

Mean pressure in systemic artery

The mean pressure in the systemic artery was not significantly changed after operation compared with the preoperative value in 18 patients, nor were there any significant changes in groups S or L.

Pulmonary vascular resistance

Pulmonary vascular resistance values during exercise were not significantly changed after operation in the whole group of 22 patients, nor in groups S or L.

In nine cases the postoperative pulmonary vascular resistance during exercise had decreased compared to resting values, and in 12 cases it had increased.

Peripheral vascular resistance

The value of the index of peripheral vascular resistance was not significantly changed either in the whole group of 25 patients or in the 16 patients in group S during exercise. Seven of the nine patients in group L had increased their values of peripheral resistance after operation, corresponding to mean increase of 159 ± 55 dynes sec cm 5 a probably significant difference.

Ventilation

The correlation between ventilation and oxygen uptake during exercise was highly significant (r — 0 86) as at the preoperative investigation There was no significant dif ference in elevation between the two regres sion lines.

To sum up the comparisons between the post and preoperative heart catheterizations the oxygen uptake was generally lower and the A—V oxygen difference larger at rest postoperatively resulting in lower mean va lues of cardiac output in all groups, but the differences between means or adjusted means of cardiac output were insignificant. During exercise at equal loads pre and post operatively there were no significant changes after operation either in A—V oxygen difference or in cardiac output. No large pre postoperative differences in stroke volume at rest were observed but during exercise a decrease of stroke volume was found be fore and an increase after operation. There was a marked decrease in pulmonary artery mean pressure and in pulmonary wedge mean pressure, generally of a high degree of significance in almost all groups post operatively In the group NI, however there were no differences of any significance and to this group belonged four of the five pa tients in the whole material who had higher pulmonary artery mean pressures after ope ration at rest Also during exercise at equal loads pre and postoperatively both pulmo nary wedge mean pressure and pulmonary artery mean pressure were lower after operation. The decrease of the latter pressure was most marked. The relative decrease in postoperative values of right ventricle work against pressure at rest was even more mark ed than that of pulmonary pressures, and the post preoperative differences were of a high degree of significance in almost all groups.

No large post preoperative differences were found in systemic artery mean pressure at rest or during exercise. A moderate mean decrease in pulmonary vascular resistance at rest of about 100 dynes sec cm^{-5} was found only in the groups I and I, during exercise there was no significant post preoperative difference either in the whole material or in groups S or L. The mean value of systemic vascular resistance at rest increased by 175—400 dynes sec cm^{-5} after operation in many groups, and only 11 of 42 patients had lower values postoperatively During exercise there was a probably significant increase of re sistance index in group L but not in the whole material or in group S.

Comments on findings at postoperative catheterization

The reduction of pressures in the pulmona ry circulation at rest was the most marked and significant hemodynamic change after opera tion in the present material. This finding agreed well with most other hemodynamic studies of the effect of valvulotomy (Eliasch, 1952, Björck et al 1953 Wade et al., 1954, Ellis et al. 1954 Lyons et al 1959) The most significant mean reduction of pressures was in group I whereas in group NI there was no significant mean change. This good correlation between decrease in pulmonary vascular pressures and functional result of commissurotomy was also in accordance with findings in other studies (Lyons et al., 1959) Wade et al. (1954) observed a fall in pulmonary pressures in every patient after operation, but the degree of reduction was not closely correlated to the clinical state after operation.

The post preoperative mean difference in pulmonary artery mean pressure during exer cise was about the same as at rest in the present study Lyons et al (1959) found a

mean reduction of 10 mm Hg in pulmonary artery mean pressure and 7 mm Hg in pulmonary artery wedge mean pressure during exercise after operation compared to preoperative exercise alone in a study of 12 patients.

It may be stated that in general most patients have less dyspnea in supine position after valvulotomy than before operation. If on that account, they are investigated in a more horizontal position postoperatively a systematic error may be introduced (Eliasch et al. 1961) in comparisons of post and preoperative pulmonary vascular pressures. In patients with mitral stenosis both pulmonary artery mean pressure and pulmonary wedge mean pressure were found to be significantly higher in horizontal position than with the trunk elevated 40° abov the horizontal plane (Cardus et al., 1958) Thus the effect of a systematic error of that kind would result in higher pressures after operation which is contrary to the findings in most studies

The changes in cardiac output at rest fter commissurotomy ha varied in different studies. In 9 patients studied by Donald et al (1957) 7—12 months after operation the resting cardiac output had decreased in seven In investigations performed about six weeks after valvulotomy analyzed by Werkö (1964) there was an increase of cardiac output at rest in almost all patients compared to preoperative values. I the present study only 11 of the whole group of 53 patients had higher resting values of cardiac output after operation and none of the eleven had also a higher oxygen uptake

The cardiac output during exercise compare to preoperatn values was increased postoperatively in five of the six patients studied by Donald et al. (1957) In the present study 12 of the 19 patients studied during exercise at the same load pre and postoperatively had a higher cardiac output fter operation, but the difference between the adjusted means of the two linear regressions of cardiac output on oxygen uptake was not significant.

A marked postoperative decrease in right ventricle work against pressure at rest was found by Donald et al. (1957) and was also observed in the present study In the study of Donald et al. the largest decrease was in patients with the best clinical results of operation. In agreement with these findings the greatest decrease in right ventricle work against pressure in the present study was in group I in patients with sinus rhythm and here was no significant postoperative change in the NI group

Donald et al. (1957) found a decrease in resting stroke volume after operation and a higher stroke olume during exercise than at rest in almost every case. Preoperatively the stroke volume increased during exercise only in one patient in the same study which included patients with sinus rhythm as well as with atrial fibrillation. These findings agree well with the results for patients with sinus rhythm in the present study In both studies the postoperative pulse rates in the investigations at rest were generally lower after operation. The lower postoperative stroke olumes at rest could therefore not be attributed to shorter filling time of the left ventricle. Thus lower filling pressure of the left ventricle at rest postoperatively did not seem to be able to maintain the preoperative level of stroke volume. During exercise, however, the stroke volume increased above the preoperative level in spite of lower pulmonary wedge mean pressure after operation.

The significant postoperative decrease in resting ventilation related to oxygen up-

Ventilation

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To sum up the comparisons between the post and preoperative heart catheterizations the oxygen uptake was generally lower and the A—V oxygen difference larger at rest postoperatively resulting in lower mean va lues of cardiac output in all groups, but the differences between means or adjusted means of cardiac output were insignificant. During exercise at equal loads pre and post operatively there were no significant changes after operation either in A—V oxygen difference or in cardiac output. No large pre-postoperative differences in stroke volume at rest were observed, but during exercise a decrease of stroke volume was found be fore and an increase after operation. There was a marked decrease in pulmonary artery mean pressure and in pulmonary wedge mean pressure generally of a high degree of significance, in almost all groups post operatively In the group NI however there were no differences of any significance, and to this group belonged four of the five pa tients in the whole material who had higher pulmonary artery mean pressures after ope ration at rest. Also during exercise at equal loads pre and postoperatively both pulmonary wedge mean pressure and pulmonary artery mean pressure were lower after operation. The decrease of the latter pressure was most marked. The relative decrease in postoperative values of right ventricle work against pressure at rest was even more mark ed than that of pulmonary pressures, and the post preoperative differences were of a high degree of significance in almost all groups.

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MITRAL AREA INDEX AND SOME CORRELATIONS

An index of mitral area $\frac{f}{\sqrt{\bar{p}}}$ (f = mean diastolic flow $\bar{p}$ = mean pressure gradient over mitral valve) was calculated as described in the chapter on methods. This mitral index was calculated in 38 patients who were investigated by combined left and right heart catheterization after operation (Table A in Appendix) The values were calculated from investigations at rest. The range of values was 30—193 Fifteen of the patients had atrial fibrillation. The mean value of the indices was 36 % higher in group S than in group L but the difference was not significant (Table 38) Four patients were classified in N.Y.H.A. group III after operation. All of them had an index less than 61 Highly significant negative correlations were found between the index of mitral area and pulmonary artery mean pressure, and also between the same index and pulmonary wedge mean pressure, $= -0.32$ and -0.54 respectively (table 39)

There were probably significant correlations both between this index and pulmonary vascular resistance at rest and between the index and right ventricle work against pressure at rest. Neither specific ventilation, relation of stroke volume to blood volume, difference between stroke volume during work and at rest, maximal work intensity nor $\frac{V_{O_2}}{\Delta t}$ in sitting or in supine position were significantly correlated to the index of mitral area.

Comments

The pressure gradient over a stenotic mitral valve has been shown to vary with the square of the flow (Gorlin & Gorlin, 1951, and Rodrigo 1953) The formula of Gorlin & Gorlin contains empirically found constants, the validity of which was discussed by Rodrigo (1953) In the present study these constants were omitted and only the relation between diastolic mean flow and square root of pressure gradient was calculated. The formula of Gorlin & Gorlin is based on the assumption of a constant volume flow over the mitral valve during diastole, and this is probably approximately true in cases with tight mitral stenosis. In the pressure recordings in the present study there was not seldom pressure difference between pulmonary wedge mean pressure and left ventricle curve only in the first part of diastole. This was found most often in cases with atrial fibrillation and with diastoles of long duration. In the latter cases it may be assumed that the main part of the volume of flow over the mitral valve is simultaneous with the recorded pressure gradient over the valve. As is described in another section, the diastolic filling time was considered to be equal to the duration of these pressure gradients in the present study

One of the errors in calculating an index of mitral valve area is the use of the mean value of flows and pressures. Correctly a mean value of the instantaneous relations between flow and square root of pressure

take was especially marked in group I in the present study and was consistent with the findings of Donald et al (1957) The latter also observed a marked decrease in ventilation related to oxygen uptake during exercise after operation. In the present study there was no significant difference of that kind during exercise. Donald et al. found a decrease in resting oxygen uptake during catheterization after operation, which they attributed to a decrease in work of ventilation. Also in the present study there was a significantly lower resting oxygen uptake at the postoperative catheterization compared to the preoperative. There was however no significant post preoperative mean change in "basal" metabolic rate in the present study so the decrease seemed better to be explained by less nervous tension in the patients during the postoperative investigations.

In most studies pulmonary vascular resistance values have been markedly decreased after commissurotomy in the majority of cases (Ellis et al. 1954 Lyons et al. 1959 Merril et al., 1961) The latter authors reported only values of total pulmonary resistance Werkö et al. (1953) observed a decrease in pulmonary vascular resistance at rest six to eight weeks after operation in 16 of 19 cases with severe pulmonary hypertension before operation. Of 13 patients with a moderate pulmonary hypertension preoperatively only two had a decrease of the resistance after operation In the present material only three of the patients who were catheterized after operation had preoperative resistance values of 800 dynes sec cm^{-5} or more (cases 31 36 55) These three patients together with case 19 had the most marked postoperative decrease of resistance in the whole material. Their postoperative values (178 410 and 298 dynes sec cm^{-5} respectively) were almost normal or slightly above the normal variation. Only a probably significant decrease of mean pulmonary vascular resistance at rest was found in some groups. Compared to resting values there was no significant postoperative change in pulmonary vascular resistance during exercise. Nor did Donald et al. (1957) find any marked uniform changes of pulmonary vascular resistance during work.

The systemic pressure at rest was measured in the brachial artery in almost all patients before operation and in the aorta in 47 of 55 patients after operation. In the post preoperative comparisons of mean pressures in the systemic artery and of peripheral vascular resistance, a systematic error is thus introduced. The mean pressure in the proximal part of the brachial artery is generally only 2 to 3 mm Hg lower than in the proximal part of the aorta. The preoperative mean pressure in the brachial artery in all patients was 84 $\pm$ 1 7 mm Hg. The systematic error was thus in general less than 5 percent. In the present study it resulted mostly in somewhat too high values of post-preoperative differences in mean pressures and in peripheral vascular resistance. The uniform postoperative increase in systemic vascular resistance at rest in the present study is too high to be markedly influenced by this error

Peripheral resistance is positively correlated to age in healthy individuals. The follow-up period was too short to cause the post preoperative changes in peripheral resistance in the present study

and pressure gradient underestimate the mitral area.

Thus after successful valvulotomies calculated areas or indices of the mitral valve would be expected to be less accurate than before valvulotomy The highest values (index >129) of mitral index were found in case 29 who had a normal pulmonary wedge mean pressure (3 mm Hg) and was classified in N Y H.A. group II after operation, in case 72 who died in bacterial endocarditis and at autopsy had a mitral valve that admitted more than one finger and in case 34 who had a pulmonary wedge mean pressure of 10 mm Hg and was classified in N Y.H A. group II. The lowest values (index <32) were found in cases 27 36 and 46 classified in N Y.H.A. groups II I and II respectively and with postoperative pulmonary wedge mean pressures at rest of 35 22 and 7 mm Hg respectively

Thus both the highest and lowest values of mitral index were found in the same postoperative groups according to N Y.H.A. The two cases with the lowest values of mitral index had postoperative values of pulmonary vascular resistance which were moderately increased above normal values (289 and 410 dynes sec cm^{-5}) These values of pulmonary vascular index were two of the three highest postoperatively

The mean diastolic mean pressure gradient over the mitral valve in these 48 patients was 8.6 mm Hg, range 2—21 mm Hg. Eleven patients had gradient above 10 mm Hg and nine below or equal to 5 mm Hg. The mean end-diastolic pressure in the left ventricle was 8.6 mm Hg, range 1—24 mm Hg Only three patients (cases 28 34, 55) had an end-diastolic pressure above 15 mm Hg.

Table 38

Postoperative index of mitral area in different groups

Groups	n	$\bar{x}$	ε	$\bar{d}$	ε_d	P
S	20	74.2	6.3	—19.7	10.77	>0.05
L	17	54.5	9.1			
I	25	63.8	6.5	+ 4.0	11.50	> 0.2
NI	13	67.8	9.9			

Table 39

Correlations of some significance between indices of mitral area and some data from combined right and left heart catheterization at rest after operation

	n	$\bar{x}$	ε	r	P
Mitral area index	37	64.6	5.5	—0.52	<0.001
Pulmonary artery mean pressure mm/Hg		21.1	1.3		
Mitral area index	37	64.6	5.5	—0.54	<0.001
Pulmonary wedge mean pressure, mm/Hg		13.2	0.9		
Mitral area index	37	64.6	5.5	—0.40	0.01—0.05
Pulmonary vascular resistance index units		1.83	0.16		
Mitral area index	38	63.1	5.4	—0.35	0.01—0.05
Right ventricle work against pressure, kpm/min		1.10	0.08		

should be used in the calculations. The validity of the formula of Gorlin & Gorlin and therefore also of the index of mitral area used in the present study is greatest in cases of tight mitral stenosis (Rodrigo 1953). After commissurotomies with good functional results there is in most studies an increased frequency of mitral insufficiency. In the present material 35 patients had palpable regurgitation immediately after commissurotomy. The calculated mean diastolic flow is a net forward flow. In cases with mitral regurgitation of any significance indices or areas calculated from mean diastolic flow

Table 40

Correlations of some significance between post preoperative differences

Post-preoperative differences	n	$\bar{x}$		P
Pulmonary artery mean pressure, mm/Hg QRS mean vector in frontal plane, degrees	36	− 14 − 12	+0.37	0.01—0.05
Pulmonary artery mean pressure, mm/Hg Max. work intensity in supine position, kpm/min	36	− 14 +106	−0.46	0.001—0.010
Pulmonary artery mean pressure, mm/Hg Max work intensity in sitting position, kpm/min	41	− 13 + 48	−0.42	0.001—0.010
Pulmonary artery mean pressure, mm/Hg Specific ventilation ml_{BTPS} ml_{STPD}	42	− 13 − 0.0029	+0.48	0.001—0.010
Pulmonary vascular resistance, units Max. work intensity in sitting position, kpm/min	40	− 0.47 + 41	−0.44	0.001—0.010

In the 25 patients with a decrease in pulmonary artery mean pressure of 10 mm Hg or more, 15 had a postoperative decrease and 6 a postoperative increase in specific ventilation. In the 18 patients with smaller decrease or an increase in the same pressure, 9 patients had a decrease and 8 an increase in specific ventilation. The correlation between change of specific ventilation and change of pulmonary artery mean pressure was significant (r = 0.48).

Thus in this study an increase in QRS mean vector in the frontal plane and in blood volume and a decrease in maximal work intensity after operation was rather seldom seen in patients with a decrease in pulmonary artery mean pressure of 10 mm Hg or more.

GENERAL DISCUSSION

Methods for evaluating postoperative results

In the present study the most significant post preoperative differences in groups with the best results of valvulotomy evaluated by the surgeon at operation (group S) and with the best clinical improvement (group I) were found in the following values QRS mean vector in frontal plane maximal work intensity both in sitting and supine position, total hemoglobin and blood volume pulmonary artery mean pressure and pulmonary artery wedge mean pressure, difference between stroke volume at rest and during work.

As reported above the postoperative decrease in pulmonary artery mean pressure was closely related to clinical improvement according to the N Y.H.A. classification. Of 32 patients with a postoperative decrease in pulmonary artery mean pressure of 5 mm Hg or more 26 were improved and of the 11 patients with a smaller postoperative decrease or with an increase in pulmonary artery mean pressure 9 were unchanged or had deteriorated. If a change in pulmonary artery mean pressure after operation is assumed to indicate the results of commissurotomy the value of an investigation for predicting this postoperative result can be judged

The change of QRS mean vector in the frontal plane was a rather good indicator of the effect of valvulotomy on pulmonary artery mean pressure. Of the 22 patients who had a postoperative decrease in pulmonary artery mean pressure of 10 mm Hg or more, only three (cases 16, 33 60) had a more positively directed QRS mean vector after operation and one had an unchanged direction of the vector Of the 15 patients with a smaller decrease or with an increase in pulmonary artery mean pressure, the direction of the mean vector was more positive in six patients, unchanged in 3 and less positive in six patients after operation. The correlation between the pre-postoperative differences in pressures and in direction of QRS mean vector was probably significant (Table 40)

A postoperative decrease in maximal work intensity in supine position was found in only one of the 21 patients with a decrease in pulmonary artery mean pressure of 10 mm Hg or more and in 5 of the other 15 patients studied in this way The correlation between pre-postoperative changes in maximal work intensity in sitting and in supine position on the one hand and pulmonary artery mean pressure on the other was significant ($r = 0.42$ and 0.46)

There was a significant negative correlation between changes of maximal work intensity in sitting position and of pulmonary vascular resistance ($r = -0.44$)

Blood volume related to body weight had increased in 4 of the 21 patients with a postoperative decrease in pulmonary artery mean pressure of 10 mm Hg or more and in 7 of the other 15 patients. There was no significant correlation between changes in pulmonary artery mean pressure and in blood volume.

Bad results

There were four patients who had gone up one N.Y.H.A. group after operation (cases 12, 20, 37 39) Two of them were males, range in age at operation was be tween 42 and 50 years Valvular heart disease had been diagnosed in two in early childhood, in the others 9 and 20 years before operation. Three had combined valvular disease (group B) three a less tight mitral stenosis (group 2) and three an adequate mitral split (group 5) Two of the patients had atrial fibrillation both pre and postoperatively three of them had some degree of palpable mitral regurgitation immediately after commissurotomy two had slight or no calcification or fibrosis of the valves. The highest value of pulmonary vascular resistance in these cases was 434 dynes sec cm 5 (case 39) the others had normal values of pulmonary vascular resistance. All of them had decrease in maximal work intensity in sitting position after operation, range of decrease 65—485 kpm min All three patients had big hearts, between 1030 and 2760 ml

Common f ndings n these four patients were thus high age (above 42 years of ge) big heart volumes and a long duration between diagnosis of valvular disease and heart operation

The importance of mitral insufficiency in evaluating postoperative results

As stated in an earlier section, no attempt was made in this study to evaluate the de gree of mitral insufficiency either before or after the heart operation. It was also stressed that predominant stenosis was diag nosed by the surgeon at operation in all cases nd that the existence or not of mitral insufficiency in this material did not seem to have significantly influenced the preopera tive results, judging from the non-significant differences between groups A and B not taking phonocardiographic findings into account.

It is important also to know to what de gree an insufficiency induced at the opera tion has influenced the postoperative results. It was not possible to ascertain this by the methods employed. From the pre postopera tive changes in heart volume some conclusions may however, be drawn. Only in 6 patients did the heart volume increase more than 150 ml after operation and the greatest ncrease was 290 ml (case 52) Except in the latter patient no increase amounted to 20 % of the preoperative value. Also in cases with induced mitral insufficiency of significance for the hemodynamics there may be a decrease in size of the right ventricle and of the left atrium after commissurotomy The relation between this decrease and the increase in size of the left ventricle following an induced insufficiency is not possible to estimate by the methods used to-day

In cases with preoperative myocardial insufficiency of the right ventricle combined with marked postoperative decrease of pul monary arterial pressure there will be a marked decrease in size of the right ventricle following commissurotomy This decrease may outweigh the increase in size of the left entricle fter an induced significant mitral regurgitation. Disregarding such cases it may be assumed that large regurgitation through the mitral valve results in an increase of heart volume. If this is true, it may be concluded from the figures above that the incidence of serious mitral insufficiencies induced at operation is too low to significantly influence the postoperative results in the present material.

Twenty patients had a decrease in pulmonary artery wedge mean pressure of 10 mm Hg or more after operation and 20 patients had a smaller decrease or an increase in that pressure. Atrio-systolic murmur had disappeared postoperatively in 7 of 8 patients who had such a murmur pre-operatively in the first group and in 4 of 8 in the second.

In the first group of patients with a decrease of pulmonary wedge mean pressure of 10 mm Hg or more two had developed a third sound after operation and four in the second group. Of the seven patients who had an increase in pulmonary wedge mean pressure after operation one had a third sound (case 48).

There was no significant correlation between changes in pulmonary wedge mean pressure and in 2—OS intervals.

The pre-postoperative changes in differences between stroke volume at rest and during work were about the same in patients with a marked postoperative decrease in pulmonary wedge mean pressure and in those with a smaller decrease in this pressure.

Analysis of cases with the best and with the worst postoperative results

The best results

In the present study there were 10 patients who fell by two N.Y.H.A. groups or more after operation. Four of them were males. The mean age in this group was 35.2 years, about five years lower than in the whole material. The mean duration of dyspnea before operation and the mean time for diagnosis of valvular disease before operation was about the same as in the whole material. Six of the patients had pure mitral stenosis (group A) and all had tight mitral stenosis (group 1) before operation. In 5 of them the valvulotomy was judged to be adequate by the surgeon (group 5). Seven of the patients had sinus rhythm and two had atrial fibrillation both pre- and postoperatively. One had atrial fibrillation before and sinus rhythm after operation.

The three patients with the highest preoperative values of pulmonary vascular resistance (cases 31, 36, 55) in the whole material were included in this group and all three had almost normal or slightly increased values after operation (178—410 dynes sec cm^{-5}). Eight of the ten patients had an increase of maximal work intensity in sitting position after operation and the mean increase was + 172 kpm/min. Two of them were not tested before operation. Mitral regurgitation of some degree was palpated immediately after commissurotomy in eight patients, in one patient the operative record contained no statement concerning regurgitation. In six of the cases rather marked calcification or rather marked fibrosis of the mitral valves was palpated by the surgeon, in one patient there was no report of the existence or not of such changes.

To summarize: The good results in this group of patients were obtained in spite of rather marked changes of the mitral valves and in spite of inadequate split of the mitral valve in five of the patients. All patients had a tight mitral stenosis before operation, and high values of pulmonary vascular resistance were rather common in this group. One of the three patients in the whole material who according to specified criteria, had myocardial insuffiency (case 55) was included in this group of ten patients. He was the only patient who had manifest edema and he had the largest heart volume in the whole material (2950 ml). His heart volume had decreased by 880 ml at the postoperative investigation and the pulmonary artery mean pressure by 25 mm Hg.

of the three patients with signs of myocardial insufficiency and in patients with marked calcification of the valves.

High pulmonary vascular resistance in combination with tight mitral stenosis seems rather to increase the indication for valvulotomy

In the postoperative examinations it was found in some cases that the effect of commissurotomy was practically annulled by factors which could be related to deficient follow-up of the operated patients. In some cases sinus rhythm had changed to atrial fibrillation, and inadequacy or absence of digitalization had brought about a high, irregular ventricular rate which rendered the patients almost as seriously disabled as before operation. In some cases a return to or onset of hypochromic anemia had diminished the oxygen-transporting capacity of the blood to such a degree that the patient s symptoms were as severe after as before operation. These findings show that in many cases regular follow-up treatment is a prerequisite for maintenance of good effect of the operation

Many patients become accustomed to physical inactivity for many years before their heart operation and hence the musculature and circulatory organs are adapted to this state. In some cases a continued physical inactivity after commissurotomy may reduce the clinical effect of an anatomically successful operation. Discrepancies between different findings from, for example exercise tests and heart catheterization may also be explained by such an adaptation to physical inactivity

The results of commissurotomy can in such cases be fully utilized through postoperative check-ups and supervision of the patient s rehabilitation.

A calculation of the number of patients who could be restored to full-time vocational work after the operation is difficult since the majority of patients in this material were housewives. If, however, N.Y.H.A. groups I and II are generally considered to be compatible with full-time vocational work, 51 patients may be calculated to have been fully capacitated at the time of the follow-up compared with 25 before operation.

Influence of varying follow up time

If postoperative *findings* change significantly with time postoperative results are a function of follow up time. No large series of operated cases with *repeated* hemodynamic postoperative studies after the first year have been reported

To judge from interindividual comparisons between results obtained after a short and after a long follow-up time in the present investigation there does *not seem to* be any important systematic change with time. Thus in the 21 patients with a follow-up time of 8—32 months the mean decrease in pulmonary artery mean pressure was 10 3 ± 2 7 mm Hg and in the 21 patients with a follow-up time of 33—59 months 15.8 ± 3 4 mm Hg The difference between the two groups was not significant.

Restenosis

According to many authors restenosis of the mitral valve after commissurotomy is seen almost only in cases with unsatisfactorily split valve (Glover et al 1955 Belcher 1959) In other studies cases have been reported in which restenosis occurred after a split of the mitral valve judged to have been satisfactory (Pattersson & Marshall, 1959 Carlotti et al. 1961 Wilhelmsen et al. 1962) In the present material there were only 3 patients who had a postoperative increase of pulmonary wedge mean pressure of more than 5 mm Hg compared to the preoperative value Two of them had myocardial insufficiency (cases 55 59) which could explain the increase in wedge mean pressure. Another of these patients (case 51) had an incomplete split of the mitral valve at operation and he was reoperated by transventricular technique some weeks *after the post* operative examination. He had a tight mitral stenosis without insufficiency and the valve was successfully split with a dilator. As repeated hemodynamic studies in individual cases have not been performed in the present study the incidence of restenosis cannot be certainly assessed. In cases with adequately split valves there were, however, none with a significantly tighter stenosis after than before operation.

Conclusions

With reference to the purpose of this study stated in section 2 the following may be concluded

I The best postoperative results were generally attained in cases judged at operation to have tight mitral stenosis and in those with valves judged to be satisfactorily split.

II Pre-postoperative change in functional state of the patients evaluated from the history of daily activities was best correlated to changes of pressures in the pulmonary circulation at rest.

III The hemodynamic changes after operation could not be predicted in the individual case by any single method. In groups of patients these changes could fairly well be predicted however by evaluating the results of exercise tests, changes in QRS mean vector in the frontal plane and in blood volume.

IV Of the different values from postoperative investigations the mitral area index calculated from postoperative catheterization values was best correlated to postoperative pulmonary artery mean pressure and pulmonary artery wedge mean pressure

The following conclusions may furthermore be drawn from the study No evidence was found for selection of patients for commissurotomy with regard to age heart size durations of symptoms etc. Good results were obtained in many of the fairly elderly patients, in patients with big hearts, in one

of the three patients with signs of myocardial insufficiency and in patients with marked calcification of the valves.

High pulmonary vascular resistance in combination with tight mitral stenosis seems rather to increase the indication for valvulotomy.

In the postoperative examinations it was found in some cases that the effect of commissurotomy was practically annulled by factors which could be related to deficient follow-up of the operated patients. In some cases sinus rhythm had changed to atrial fibrillation, and inadequacy or absence of digitalization had brought about a high, irregular ventricular rate which rendered the patients almost as seriously disabled as before operation. In some cases a return to or onset of hypochromic anemia had diminished the oxygen-transporting capacity of the blood to such degree that the patient's symptoms were as severe after as before operation. These findings show that in many cases regular follow-up treatment is prerequisite for maintenance of good effect of the operation.

Many patients become accustomed to physical inactivity for many years before their heart operation and hence the musculature and circulatory organs are adapted to this state. In some cases a continued physical inactivity after commissurotomy may reduce the clinical effect of an anatomically successful operation. Discrepancies between different findings from, for example, exercise tests and heart catheterization may also be explained by such an adaptation to physical inactivity.

The results of commissurotomy can in such cases be fully utilized through postoperative check-ups and supervision of the patient's rehabilitation.

A calculation of the number of patients who could be restored to full-time vocational work after the operation is difficult since the majority of patients in this material were housewives. If, however, N.Y.H.A. groups I and II are generally considered to be compatible with full-time vocational work, 51 patients may be calculated to have been fully capacitated at the time of the follow-up compared with 25 before operation.

SUMMARY

The subject for this dissertation is a clinical and hemodynamic study prior and subsequent to commissurotomy in a series of 60 patients with mitral stenosis. The patients selected for the study were operated upon at the Thoracic Clinic of Karolinska sjukhuset during the years 1957—1960 and underwent a complete heart examination including right heart catheterization before operation. Fifty-one of the patients had come to the follow-up in response to our request, the other nine had been referred to the hospital for various reasons. The proportion of females to males was 2.3:1 and the average age at heart operation 40 years. The average follow-up period was 34.8 months.

The patients were followed up by the same standardized methods as in the preoperative heart examination. Furthermore combined left and right heart catheterization was done in 38 cases. In addition to ordinary bedside examination, anamnesis and the usual tests, the following examinations were made: phonocardiography, electrocardiography, exercise tests on a bicycle ergometer in sitting and supine positions, roentgenological heart volume determination in supine position, determination of total hemoglobin by the alveolar carbon monoxide method, calculation of blood volume, oxygen uptake at rest by the Krogh and Douglas methods, spirometry including determination of functional residual capacity by the helium dilution method, heart catheterization and determination of cardiac output by the direct Fick method, and pressure recording at rest and during exercise. The validity of the methods is discussed and the reproducibility of most of them is reported.

Preoperative findings

No patient was confined to bed before operation, only one had manifest peripheral edema. The grouping of the patients according to the New York Heart Association (N.Y.H.A.) classification was: Group I—6, II—19, III—29, IV—6. Twenty-one patients had atrial fibrillation at the time of the heart operation. Forty-nine were on digitalis therapy. In 10 there was no distinct opening snap and in 6 no apical diastolic murmur on the phonocardiogram; 3 lacked both a diastolic murmur and opening snap.

On the electrocardiograms the P and QRS vectors were calculated in the frontal plane after measurement of the relevant areas in the extremity leads. As a measure of the QRS vector in the horizontal plane a corresponding measurement was made of the difference between the areas in CR_7 and CR_2. Electrocardiographic signs of right ventricular hypertrophy were judged according to criteria proposed by Milnor (1957). The mean values of the P and QRS mean vectors in the frontal plane were within normal variations reported in the literature when the vectors were calculated in the same way. Eight of the patients fulfilled the criteria for right ventricular hypertrophy.

The mean value of maximal work intensity in sitting position was for men 504 ±

50 kpm/min and for women 333 ± 19 kpm/min with somewhat higher values in cases of sinus rhythm than of atrial fibrillation, though the difference was not significant. The corresponding values in supine position were for men 332 ± 30 kpm/min and for women 195 ± 18 kpm/min. Again the difference between patients with sinus rhythm and with atrial fibrillation was not significant.

The mean heart volume in supine position for men with sinus rhythm was 1096 ± 38 ml and with atrial fibrillation 2026 ± 348 ml, for women 873 ± 31 ml and 1134 ± 60 ml respectively The difference in heart volume between patients with sinus rhythm and with atrial fibrillation was highly significant both for men and women.

The mean value of total hemoglobin in g/kg body weight was 9.5 ± 0.3 in women and 10.2 ± 0.4 in men. The mean blood volume in ml/kg body weight was in women 84.0 ± 2.5 and in men 82.9 ± 2.4. The mean hemoglobin concentration was 12.2 ± 0.3 g % in women and 13.1 ± 0.3 g % in men. The values of total hemoglobin and of blood volume in men did not differ significantly from the normal values found by the same method in the laboratory

Vital capacity functional residual capacity and total capacity were highly significantly lower than the predicted values and the quotient between residual volume and total capacity highly significantly higher

A significant correlation existed between oxygen uptake and cardiac output at rest in patients with sinus rhythm but not in patients with atrial fibrillation. The cardiac output of patients with sinus rhythm was significantly lower 1.3 l/min, than in a normal material studied at the laboratory The cardiac output of patients with atrial fibrillation was significantly lower than in patients with sinus rhythm. The mean value of the pulmonary arterial mean pressure at rest was 34.3 ± 1.8 mm Hg of the pulmonary wedge mean pressure 21.8 ± 0.9 mm Hg, and of the work of the right ventricle against pressure 2.2 ± 0.1 kpm/min. The mean resistance in the pulmonary vessels was 221 ± 30 dynes sec cm^{-5}

During exercise there was a highly significant positive correlation between oxygen uptake and cardiac output the regression equation with cardiac output (l/min) as dependent variable and oxygen uptake (ml/min) as independent variable was $y = 0.007x + 2.44$. The correlation between these variables was still higher in patients with sinus rhythm (= 0.821) During exercise the stroke volume fell significantly below the resting level. A highly significant correlation existed also between ventilation (l/min) and oxygen uptake (ml/min) during exercise the regression equation was $y = 0.0217x + 7.2$ with ventilation as dependent variable and oxygen uptake as independent.

On the basis of their pre-postoperative status according to the N.Y.H.A. classification the patients were grouped as improved and not improved, with 40 patients in the former and 20 in the latter group. They were also grouped according to the surgeon's evaluation of the tightness of the mitral orifice before and after commissurotomy also as pure mitral stenosis and mitral stenosis combined with other valvular defects, the latter grouping on the basis of the phonocardiographic findings and the surgeon's palpation findings before the commissurotomy According to the preoperative heart examination and the surgeon's findings at operation there was no patient with predominant mitral insufficiency Forty-two patients were classified as tight preoperative mitral stenosis

(mitral orifice<one finger) and 17 as less tight In 25 cases there were signs of mitral insufficiency before operation No attempt was made in this study to evaluate the degree of insufficiency Thirty two patients had pure mitral stenosis before operation, the other 28 combined valvular defect, 19 of whom a combined mitral valvular defect and 9 a combination of aortic and mitral defect In 29 cases the surgeon found at the operation fibrosis and/or calcification of the mitral valves of serious degree, in 22 cases no or only mild lesions of this nature.

In 35 of the cases the surgeon judged the commissurotomy to be satisfactory that is to say that the mitral orifice was judged to admit two fingers after the operation in 24 cases the mitral orifice was judged to be less than two fingers in width after the operation. In all cases digital commissurotomy was performed in the first place, in 30 cases being supplemented by transventricular dilation of the mitral orifice with a dilator

The incidence of mitral insufficiency in the tight and less tight stenosis groups was not significantly different from the incidence for the entire material. The incidence was probably significantly higher in the groups with pronounced valvular fibrosis and/or calcifications.

According to certain defined criteria three patients were judged to have myocardial insufficiency before the operation.

In some of the preoperative examinations there were significant differences between the tight and less tight mitral stenosis groups. Cases with less tight stenosis, for example, had significantly higher mean values of maximal work intensity in sitting position, and of cardiac output and stroke volume. They also had lower pulmonary arterial pressure and pulmonary wedge pressure.

Postoperative findings

Of the 60 patients examined and of the 10 patients who could not come to the follow up, 4 had died at the time of the follow-up one of whom 15 days after the heart operation. This represents a late mortality of 4—5 percent.

Seven patients who had earlier had sinus rhythm had developed atrial fibrillation during the follow-up period, and two who had earlier had atrial fibrillation had reverted to sinus rhythm.

The appearance of a third sound and prolongation of the interval between the second sound and opening snap (2—OS interval) were related to a satisfactory result of operation

In the whole material there was a highly significant lowering of the mean QRS vector in the frontal plane by 12.8 ± 2.9 degrees. In the improved group the vector was likewise highly significantly lowered, by 17.7 ± 3.8 degrees, while in the not improved group there was no significant change

In the whole material the maximal work intensity in sitting position showed a probably significant increase by 58 ± 22 kpm/min In patients with sinus rhythm both pre and postoperatively the mean increase was highly significant, 86 ± 30 kpm/min, in patients with atrial fibrillation pre and postoperatively there was no significant difference.

The highest increase in mean maximal work intensity was in the improved group 116 ± 22 kpm/min, in the not improved group there was no significant difference The changes of maximal work intensity in supine position were analogous to those in sitting position, with significant differences in the same groups. A highly significant increase of the quotient between work inten-

sity and increase of heart rate from rest to exercise in patients with sinus rhythm was observed both in the whole material and in patients with tight mitral stenosis before operation, also in patients whose commissurotomy was judged satisfactory by the surgeon at the time of operation and in the group of improved patients. Thus the changes in the work intensity — pulse rate ratio largely followed the changes in maximal work intensity

No significant changes of heart volume were noted. The total hemoglobin had diminished by an average of 0 7 ± 0.2 g/kg body weight. There were no significant differences in hemoglobin concentration. The mean blood volume for the whole material had diminished by 5 9 ± 1 9 ml/kg body weight.

An increase f functional residual capacity in relation to predicted values was the only highly significant change in the spirometric values

As in other similar studies, there were no significant differences in cardiac output at rest postoperatively There was a probably significant reduction of stroke volume by 5.8 ± 2 7 in patients with sinus rhythm. A highly significant reduction of the pressures in the pulmonary artery and of the pulmonary wedge pressure was noted in the whole material (12 6 ± 2.2 and 8.4 ± 1 4 mm Hg respectively) Significant reductions in these pressures were noted also in the majority of the separate groups. A significant reduction of the right ventricle work against pressure was recorded in the entire material, 1 1 ± 0.2 kpm/min, and also in the majority of separate groups. The index for pulmonary ascular resistance in the ent re material was not significantly changed. It was probably significantly reduced in the group of patients with sinus rhythm the resistance index was in general most lowered in cases with high preoperative values.

There were no significant changes of cardiac output during exercise postoperatively The stroke volume, which preoperatively had fallen by 9.3 % during exercise compared with the resting value, increased after operation by 16.6 % a highly significant post-preoperative difference. The pressure in the pulmonary artery during exercise at the same loads pre and postoperatively was highly significantly lower, 14.8 ± 3.8 mm Hg, after operation, the lowering of the pulmonary wedge pressure for the whole material was 10.8 ± 3 9 mm Hg, a probably significant difference. Only three patients had a higher pulmonary arterial pressure during exercise and four a higher pulmonary wedge pressure during exercise postoperatively There was no significant difference between ventilation related to oxygen uptake during exercise pre and postoperatively

Combined right and left heart catheterization was done after operation in 38 cases. The mean pressure gradient across the mitral orifice was calculated planimetrically and from the mean pressure gradient and diastolic mean flow an index of the size of the mitral orifice was calculated. The mean value of this index was larger in patients with satisfactory than in those with unsatisfactory commissurotomy but the difference was not significant. There was a highly significant correlation between this index, on the one hand, and the pulmonary arterial and pulmonary wedge pressures on the other No significant correlation existed between this index and the maximal work intensity in supine or sitting position. Nor as there significant difference between the mean values of the indices in the improved" and not improved groups.

Methods for evaluating postoperative results are discussed. The correlation between the pre postoperative differences in pulmonary artery mean pressure and in direction of QRS mean vector in the frontal plane was probably significant ($r = +0.37$) and between the same pressure and maximal work intensity in *sitting* and *supine position* significant ($r = -0.42$ and -0.46 respectively) The correlation between pulmonary arterial mean pressure and specific ventilation was also significant ($r = +0.48$) An increase in QRS mean vector in the frontal plane and in blood volume and a decrease *in maximal work intensity after operation* was rather seldom seen in patients with a decrease in pulmonary artery mean pressure of 10 mm Hg or more

Cases with the best and with the worst clinical operative results are analyzed. The clinical results were evaluated from N Y H.A. *grouping before and after operation*. All patients with the best postoperative results had tight mitral stenosis before operation, some of them had the highest values of pulmonary vascular *resistance in the whole* material mitral regurgitation was palpated after commissurotomy in 8 of the 10 patients. Many had fibrosis and/or calcifications of the mitral valves of marked degree

The 4 patients with the worst postoperative results had all big hearts and three had *combined valvular disease*. Three of them had normal values of pulmonary vascular resistance. The age at operation of these three patients was rather high, between 42 and 50 years and there was a long duration between diagnosis of valvular disease and heart operation.

Good results were obtained in many of the fairly elderly patients, in patients with big hearts, in one of the three patients with myocardial insufficiency and in patients with marked calcifications of the valves. No evidence was thus found for selection of patients for commissurotomy with regard to these variables.

The importance of regular postoperative check-ups and supervision of the patients rehabilitation is stressed

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APPENDIX

Table A.
Data from combined left and right heart catheterization after operation

Case no.	Diastolic mean flow () ml.	Diastolic mean pressure gradient ($\bar{p}$), mm Hg	Index of mitral area $\frac{f}{\sqrt{\bar{p}}}$
1	175	16	45.0
4	171	7.0	64.7
5	211	20.3	46.3
6	202	8.8	68.0
8	210	4.0	105.0
9	150	2.5	94.8
10	152	5.7	63.6
12	185	9.6	59.6
13	152	11.2	45.3
14	140	8.6	47.8
15	191	12.0	55.2
16	174	5.8	71.4
18	137	14.9	34.6
20	102	4.6	47.4
21	151	21.3	32.7
22	157	12.5	33.8
23	107	3.2	58.1
24	254	6.6	93.9
26	122	9.3	40.0
27	113	14.5	79.6
28	117	6.0	47.0
29	237	1.5	193.0
31	154	3.5	82.3
32	109	10.5	33.6
33	149	7.8	53.4
34	279	4.6	130.0
36	136	18.3	31.4
39	171	4.2	83.4
40	216	7.5	77.7
45	189	8.8	63.6
46	79	6.2	31.6
48	224	6.9	83.2
50	133	6.1	62.7
51	146	12.0	42.2
52	167	7.6	60.5
60	227	6.9	78.6
55	121	6.0	49.4
72	297	5.1	132.8

Data on 60 patients with mitral stenosis before and after commissurotomy

Case no	Sex	Height, cm	Weight, at preoperative investigation	Body surface, m²	Age at operation, years	Preoperative history: Rheumatic fever, years before operation	Dyspnoea on exertion, years before operation	Diagnosis of valvular disease, years before operation	Atrial fibrillation, years before operation	First attack of pulmonary edema, years before operation	First haemoptysis, years before operation	Systemic embolism, years before operation	Heart rhythm at preoperative investigation	Preoperative phonocardiographic findings: Presystolic apical murmur	Diastolic apical murmur starting at O.S.	Diastolic apical murmur starting at third sound	Aortic systolic murmur	High frequent regurgitation murmur over heart base	Ejection murmur of ... over heart base	Q–I interval, seconds	2–OS interval, seconds
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE B

Other diseases than heart disease in the material

Cases	Diseases	Comments
5	Chronic hypochromic anemia	Concentration of hemoglobin 9—11.5 g % since 1957
10	Sarcoidosis	Enlargement of hilar nodes diagnosed 1958. Diagnosis confirmed by biopsy
11	Hypertension	Blood pressure varying between 170/95 and 220/120 mm Hg since 1957
25	Chronic renal disease	Treated with gold salts for rheumatoid arthritis 1951. Renal disease diagnosed 1958. Proteinuria, slightly increased blood urea nitrogen and moderately decreased clearance values since 1958
26	Pulmonary tuberculosis	Pneumothorax 1943—1947
30	Diabetes mellitus	Diagnosis 1957. Well regulated with a small dose of protamin zinc insulin and diet
36	Residual liver disease	Viral hepatitis 1947. Pathological liver function tests since 1947
52	Residual liver disease	Viral hepatitis 1961. Pathological liver function tests 1961—1962
54	Rheumatoid arthritis	Diagnosis 1962
57	Hypertension	Blood pressure varying between 180/105 and 200/120 mm Hg since 1957

Data 60 patients with mitral stenosis before and after commissurotomy

Case no.	Sex	Height, cm	Weight, at preoperative investigation	Body surface, m²	Age at operation, years	Preoperative history: Rheumatic fever, years before operation	Dyspnea on exertion, years before operation	Diagnosis of valvular disease, years before operation	Atrial fibrillation, years before operation	First attack of pulmonary edema, years before operation	First haemoptysis, years before operation	Systemic embolism, years before operation	Heart rhythm at preoperative investigation	Preoperative phonocardiographic findings: Presystolic apical murmur	Diastolic apical murmur starting at O.S.	Diastolic apical murmur starting at third sound	Aortic systolic murmur	High frequent regurgitation murmur over heart base	Ejection murmur of stenosis type over heart base	Q–I interval, seconds	II–OS interval, seconds
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

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54	Rheumatoid arthritis	Diagnosis 1962
57	Hypertension	Blood pressure varying between 180/105 and 200/120 mm Hg since 1957

Case no.	…ence values: …functional residual capacity FR (l BTPS)	Residual volume l BTPS (RV)	Total capacity l BTPS (TC)	Vital capacity l BTPS (VC)	FRC/TC, %	RV/TC, %	Arterial oxygen saturation, %	A-V oxygen difference, ml/l	Oxygen uptake, ml STPD/min	Cardiac output, l/min	Stroke volume, ml	Stroke volume/Blood volume, ml/l	Mean pressure in right atrium, mm Hg	End-diastolic pressure in right ventricle, mm Hg	Pulmonary artery mean pressure, mm Hg	Pulmonary arterial wedge mean pressure, mm Hg	Right ventricle work against pressure, kgm/min
	Differences between predicted and observed preoperative values						Preoperative catheterization at rest										
1	+0.23	+0.49	1.64	1.44	−11	2	94	40.7	230	5.7	73	18.1	3	3	19	27	2.30
[illegible]	+0.03	+0.66	+0.72	−0.04	−2	+11	96	37.5	251	6.7	98	18.1	−2		19	16	1.84
[illegible]	−0.08	−0.08	+0.17	+0.06	3	−2	[illegible]	37.1	200	5.7	96	12.8	6	6	1	23	2.08
[illegible]	+0.02	+0.23	[illegible]	[illegible]	7	−1	100	50.5	270	5.	91	2.8	5	3	26	18	1.77
[illegible]	+0.12	0.01	+0.34	+0.27	7	−2	98	35.1	257	7.3	159	16.6	2	2	38	1	2.70
7	−0.32	−0.21	+0.26	−0.09	2	−1	98	46.1	200	2.8	97				32	22	3.02
8	1.04	+0.25	+0.05	0.05		1	98	27.4	254	8.3	64	13.2	3	11	34	19	3.04
9	+0.27	0.54	[illegible]	0.02		7	102	30.8	105	4.9	92	17.8	3	4	25	23	1.30
10	+0.04	0.72	1.42	1.19	5	−3	81	47.8	240	5.2		11.8		2	32		2.25
11	−0.25	−0.56	+0.53	+1.12	8	17	95	83.1	231	4.2	—		2	2	22	21	1.70
12	1.53	1.5	1.82	1.36	2		96	36	294	8.1	100	13.2		7	25	18	2.57
13	+0.56		+0.07	+0.08	7	—	100	47.9	213	4.5	53	14.3		8	39	31	2.66
14	+0.61	+0.28	+0.17	+0.14	7	+	94	40	23	6	—		3	5	39	31	2.23
15	+0.05	−0.27	−0.35	0.5	1		95	32.0	282	8.8	71	14.2	1	1	44	71	3.43
16	+0.38	+0.05	[illegible]	1.71	6		97	41.8	244	6		11.7	2	3	26	29	3.34
17	−0.3	−0.7	.7	+0.00	3		9	38.1	259	6					78	30	2.36
18	+0.25	+0.11	0.87	+0.03	6		91	40.6	270	6.1	37	11.0	8	13	47	30	1.67
19	[illegible]	+0.11	1.22	1.2	2	6	97	42.7	305	4.8			4		21	24	2.83
20	+0.85	+0.55	0.96	+0.36	4	6	93	40.9	333	5.1	59	12.6	3	6		29	1.06
21	−0.15	−0.58	+0.00	1.00	18	—	98	83.3	225	4.2	—	—	0	3	30	27	1.82
22	+0.06	−0.58	+0.70	+1.21		18	93	43.7	219	3		—	3	6	30	1	1.7
23	+0.57	+0.00	+0.04	−0.78			93	36.5	46		22	11.0	7		41	31	2.
24	+0.05	0.50	93	+0			93	30.1	22	4					40	76	2.32
25	−0.05	−0.15	+0.06	1.06	12		93	35.6	221		30	13.1	2		34	13	2. 7
26	1.00	+0.5	1.02	−1.32		+1	93		205	5				8	33	25	2.04
27	+0.70	+0.23	+0.37	+0.14	18		93	43.5	23	5.2		—	6	10	39	26	2.53
28	+0.30	−0.40	79	1.2	7	16	95	46	207	4.5	60	16	3	3	50	1	2.45
29							95	36	205				3	3	23	32	1.94
30	+0.07	−0.14	44	1.47			98	46.5	205	.2	64	12.5			43		4.10
31	+0.03	−0.57	1.12	71			93	33.8	264	0	42	—	1	6	83	33	1.34
32	−0.70	[illegible]	−0.11	[illegible]		5	93	30.2	302	5.6		—			30	18	1.77
33	+0.01	0.2	[illegible]	[illegible]		+	95	37.8	304	4.5					34	23	1.05
34	−0.40	−0.11	+0.42	[illegible]			95	31.1	210	6.6	86	7.9		12	25	19	2.08
35	+0.01	+0.14	1.70	[illegible]		13	97	34.2	140	2.9	30	6.4			63	31	2.1
36		+0.12		1.36			95	44.4	283		44	10.2	1	5	41	33	1.82
37	0.01	+0.23		+0.70			99	32.8	245	7.5	44	15.6				33	2.76
38	0.7	−0.23	1			19	97	37	291		44	11	3	6	31	—	1.63
39				+0.04			96			7					24	17	1.87
40	−0.37	[illegible]	[illegible]				96	.2	303		96	10.0					
41	+0.33	−0.18	92			1	98	70	218	3.1	—	—		10	38	23	1.34
[illegible]	−0.70	+0.00					95	73	224	3.	72	14.7	10	2	40	30	1.02
[illegible]	−0.43	+0.12	72			1	95	40	180	4.5	76	1.6	2		19	16	1.00
44	2.88		1			10	90	1.0	243	5	47	10.0	8	5	7	3	2.00
45	+0.00	+0.15				3	96	62.3	210		—		4	2	19	16	0.02
46		+0.01	+0.73				93	39.2	190	3	85	13.7	2	8	36	13	0.00
[illegible]			+0.18		12	2	98	38.6	231		140		1		38	17	2.15
48		56					8	34.1	237	6.7	132	21	4		7	10	1.30
49						10	89	36.9	266	6	46	11.5			7	17	1. 1
50	+0.39	27	76					62.6	265						34	25	1.84
51		72	6	4.2		6	99	40.8	243	6.0	76	5.6	3	5	24	18	1.05
52		97	0.30	−0.36		1	97	44	194				3	3	17	8	0.00
53		07		.02		14	92	80.1	208	2.6	23	6.2	5	6	38	23	1.08
54		73	+0	3		3	96	64.3	303	5.5	68	13	1	8	62	30	1.57
55						8	98		278	3.3			24	2	2	13	1.08
56		−0.63	−0.18	0.07			96	36.5	238	7.7	85	22.6	2	2	23	18	2.1
[illegible]		01	−0.78	−0.05				42	305	6.3			3		40	1	2.06
58		1.22	+0.15			5	105	1.0	47		64	13.3	3	5	27		2.23
59		4.2	4			8	94	36.8	372	3.5				5	30		11
60			0.92			16	97	33	344	6	77	—				20	.45
61							94	77.3	234	1	—	—	10	1	34	25	1.43
72		300	−0.05	+0.91	−0.08	7	94	44	209		60	11.0			30	29	1.08

Case no	P-vector in frontal plane degrees	QRS-vector in frontal plane, degrees	Net area, CR − CR, mm^2	Right ventricular hypertrophy	Maximal work intensity extrapolated, in sitting position, kpm/min	Maximal work intensity performed, in sitting position, kpm/min	Six minute frequency on final load in sitting position, beats/min	Maximal work intensity extrapolated in supine position, kpm/min	Maximal work intensity performed in supine position, kpm/min	Six minute frequency on final load in supine position, beats/min	$\frac{W}{\Delta f}$ in sitting position, kpm/min/pulse beat	$\frac{W}{\Delta f}$ in supine position, kpm/min/pulse beat	Heart volume in supine position, ml	Total amount of haemoglobin, g/kg bw	Blood volume ml/kg bw	Hb-concentration, g/100 ml
Preoperative ecg-findings					Preoperative bicycle ergometer tests								Preoperative values			
1	+60	—	+14		435	400	120	300	300	114	6.2	6.8	815	8.8	76.6	11.5
2	+52	+65	+1	+	900	900	270	600	600	136	8.6	8.4	990	11.0	78.7	11.0
4	+75	+67	+28		200	200	142	100	100	126	5.1	2.9	830	9.6	83.4	11.5
5	+50	+72	+43		300	300	108	200	200	90	5.9	—	1 030	10.0	81.0	12.3
6	+60	+60	+16		465	400	139	400	400	152	5.8	5.0	905	8.5	79.1	10.7
7	+60	+77	+46		400	300	92	300	300	94	—	—	1 125	8.3	78.2	11.8
8	—	+88	+10		465	400	158	265	200	157	4.4	5.0	680	9.0	129.3	7.6
9	+30	—	+24		265	200	130	200	200	126	5.0	4.8	1 050	7.3	62.8	11.6
10	−18	−10	+18		400	400	168	150	200	140	5.0	1.9	720	8.6	74.7	11.5
11	—	+52	+27		235	200	113	200	200	116	—	—	1 165	7.8	62.8	11.6
12	+30	−10	+10		650	600	144	450	300	120	7.7	9.4	1 090	11.1	91.8	11.7
13	+51	+47	+23		335	200	102	100	100	124	5.4	2.8	820	9.2	84.3	11.5
14	—	+83	+29		335	200	148	200	200	158	—	—	1 020	7.9	86.6	11.9
15	+60	+74	+21		535	400	144	600	600	170	9.1	7.4	840	8.7	80.4	12.1
16	+51	+74	+36		365	400	148	335	200	128	4.9	4.3	1 015	9.9	84.8	11.7
17	—	+51	+36		400	400	183	200	200	148	—	—	1 140	9.6	80.4	11.9
18	+60	+80	−3		200	200	132	200	200	140	4.3	4.2	1 240	8.6	70.7	12.2
19	—	+60	+31		515	400	128	—	—	—	—	—	1 000	8.8	87.8	10.0
20	—	+80	+9		465	400	146	300	300	128	6.3	7.1	1 030	8.0	65.2	12.3
21	—	+60	+10		200	200	168	65	65	170	—	—	1 080	9.8	111.1	8.8
22	—	+90	+14		400	400	150	400	200	176	—	—	780	9.4	80.4	11.7
23	—	+77	+36		200	150	156	65	65	140	—	—	860	10.5	81.8	13.0
24	+47	+16	+14		265	200	126	200	200	118	4.9	4.3	940	8.2	72.7	11.3
25	—	+118	±0	+	265	200	162	200	200	151	—	—	1 680	9.4	74.8	12.8
26	+30	+60	+27		265	200	122	100	100	100	3.8	2.5	575	8.9	93.0	10.6
27	—	+60	+28		265	200	139	235	200	130	—	—	1 180	8.7	72.0	12.1
28	—	+90	+19		200	200	136	100	100	136	—	—	1 500	11.3	96.0	11.8
29	+30	+60	+28		335	200	124	200	200	128	5.1	3.3	890	7.8	70.2	11.1
30	+60	+81	+6	+	600	600	152	335	200	114	7.7	7.1	1 150	9.8	80.8	12.1
31	+60	+118	+1	+	300	200	172	65	0	—	2.8	—	710*	—	—	—
32	—	+90	+7		700	600	128	400	300	90	—	—	1 380	8.6	72.4	11.8
33	—	+41	+12		265	400	133	—	—	—	—	—	1 300	10.4	89.6	11.8
34	+60	+74	+19		400	400	130	—	—	—	3.5	—	725	9.6	83.2	11.3
36	+80	+120	−14	+	100	100	150	100	100	136	1.8	1.4	1 010	8.4	91.8	9.2
37	+43	+83	+29		315	300	154	200	200	125	6.1	5.1	730	10.7	83.3	12.8
38	—	+76	+32		600	600	172	—	—	—	6.7	—	850	—		—
39	—	+90	+22		300	300	170	150	150	122	3.2	3.6	775	7.6	62.8	12.1
40	−60	+54	+36		665	600	152	—	—	—	7.3	—	1 180	8.4	77.2	10.8
41	—	—	+28		115	100	139	115	100	138	—	—	1 295	11.3	100.5	11.2
42	—	+60	+8		250	200	102	225	150	144	—	—	1 740	11.2	81.8	13.7
43	+30	+80	+30		265	200	116	335	200	120	3.6	3.8	695	10.3	93.3	11.0
44	+60	+105	+31		335	200	144	65	0	—	3.1	—	1 175	11.4	—	—
45	—	—	+23		465	400	149	135	0	—	6.3	—	1 075	9.5	82.1	11.5
46	—	—	+25		330	300	118	215	200	108	—	—	1 290	13.2	100.4	13.1
47	—	+68	+25		235	200	95	225	200	113	—	2.5	880*	10.8	98.0	11.0
48	+60	+78	+23		665	600	156	600	600	107	7.4	6.1	1 000*	—	—	
49	±0	+70	+17		635	600	146	135	0	—	6.3	—	1 230	10.3	85.4	12.1
50	+60	+90	—		515	500	182	100	100	133	4.5	3.3	750	9.0	75.6	11.9
51	+60	+85	+41		700	600	159	450	600	156	8.1	7.3	885	10.4	84.2	12.3
52	—	+80	+25		300	300	152	300	300	150		—	820	10.8	87.8	12.3
53	+60	+120	—	+	—	—	—	65	0	—		—	750*	11.2	86.7	12.9
54	—	+70	+25		450	450	162	300	300	128	5.4	6.5	1 160	9.9	86.5	11.4
55	—	+114	+2	+	—	—	—	—	—	—		—	2 850	11.6	102.0	11.4
56	+60	+80	+39		600	600	163	315	300	127	5.4	5.8	1 070	—	—	—
57	—	+89	+34		470	400	135	235	200	118		—	1 200	6.9	57.3	12.8
58	+15	+80	+49		300	500	136	115	100	108	3.5	4.2	855	8.0	89.7	9.0
59	—	+80	+35		300	300	131	100	0	—		—	2 760	11.5	—	—
60	+60	+82	+7	+	470	400	130	465	400	132	5.7	7.7	1 215	—	—	—
62	—	+85	+40		165	100	138	65	—	—			1 200	13.9	120.6	11.3
72	+60	+80	+20		300	300	138	100	100	104	7.0	5.0	985	8.2	88.7	9.2

*x-ray in erect body posit

Tabl continued

Case no.	Heart rhythm at postoperative [illegible]	Post-operative changes: Appearance of [illegible]	Appearance of [illegible]	Degree of [illegible]	Q [illegible] interval, seconds	QRS interval, seconds	Vector in frontal plane, degrees	QRS-vector in frontal plane, degrees	Net area QRS, [illegible]	Maximal work intensity, [illegible] kpm/min	Maximal work intensity, [illegible] kpm/min	W/V [illegible] kpm/ml	W/V [illegible] kpm/ml	Heart volume, ml	Total amount of haemoglobin, g	Blood volume, ml/kg	Vital capacity ratio observed/predicted	Residual volume (RV) [illegible]	Functional residual capacity (FRC) [illegible]
1	f	+			−1	+2	—		+ 8	−135	−100	—	—	—	−0.6	−10.1	+0.04	+0.59	−0.09
2			+		3	−1	+	17	− 3		0	+3.1	+1.5	− 40	−1.1	− 6.0	0.06	+0.[illegible]	+0.64
[illegible]	f	+	+	+	−2	0	+13	−17	+1	+100	+115	+3.6	+1.1	− 70	−1.3	−12.0	−0.0[illegible]	+0.12	+0.27
5	1	+			+	0	—	−12		33	− 35	—	—		−0	− 6.9	+0.0[illegible]	−0.27	−0.36
6					+1	1	0	−10	− 8	+135	+100	−0.1	+0.5	15	−0.3	−10.4	−0.11	+0.33	+0.25
7	1	+			0	0		+ 9	+18	+400	300	—	—	—	+1.8	+ 2.7	+0.02	−1.00	−0.29
8				+	+3	1	—	− 8		− 65	+135	+0.3	+1.8	−130	0	−27.0	−0.05	+0.38	+0.31
[illegible]						+1	—	—	− 1	+ 35	+200	−1.2	+0	− 30	0.2	+10.4	+0.12	+0.42	−0.24
10				+	—	+4	+70	2	+ 1	0	+ 85	+2.2	+2.9	−375	1.6	−13.5	−0.10	−0.56	+0.35
11	f		+		+1		—	+ 2	+38	80	− 65	—	—	+125	+0.5	− 1.3	+0.08	−0.50	−0.27
12					+1	+2		+1	12	−485	−450	—	—	+190	−0.2	−12.1		+0.31	+0.74
13			+	+	+1	0	− 1	−19	11	+ 65	300	−1.4	+1.9	+100	−1.2	−11.8	+0.10	+0.38	+0.25
14	1				3	—		−13	+ 2	− 35	+ 35	—		0	+0.6	+13.3	—	0.45	+0.34
15					0	−1		− 3	8	+ 65	85	0	−0.7	+ 40	—	.7	0.04	0.67	+0.54
16				+	—	+1	+	+18		385	+385	+0.2	+4.3	−415	−2.0	−23.5	+0.08	+0.28	+0.54
17	1				0	1		−15			+ 65			210	−0	− 9.3	0.08	+0.10	−0.49
18					+2	—		−12	+ 7	+380	+230	—	—	—	+1.2	+ 3.0	−0.03	+0.26	+0.44
19		+			0	—		+13	+14	215	—	—	—	+140	1.6	−23.1	−0.07	+0.08	−0.07
20	f				2	1		0	− 2	− 65	0	—	—	—	+1.9	+ 1.6	+0.22	+0.29	+0.35
21	f	+			+5	0			+12	15	+130	—	—	+100	+0.8	23.9	−0.04	−0.25	−0.15
22					−1	0			+	100	0	—	—	—	−1.0	− 7.5	+0.06	−0.0[illegible]	+0.[illegible]
23					3			25	+	130	+135	—	—	—	−0	+11.0	−0.13	+0.2	+0.[illegible]
24					−4	—		11	25	+135	+300	+1.8	2.5	30	−1.2	− 8.4	−0.11	+0.06	+0.[illegible]
25	1		+		1		—		24	+ 70	+100	—	—	+ 30	+1.5	+ 8.0	−0.07	+0.18	+0.4[illegible]
26							30		− 7	0	+130	−1.3	+2.6	+125	+0.1	+ 2.1	+0.07	+0.05	+0.5[illegible]
27							—		+10	+ 70	+ 85	—	—	+220	+0.3	+ 0.7	+0.0[illegible]	+0.41	+0.5[illegible]
28					—	+	—	−40	+		300	—	—	300	2.1	64.4	+0.08	+0.45	+0.44
29										− 35		−0.6	−0.4	130	1.1	0	+0.03	—	—
30								+18	+70		+385	+9.8	+2	85	−0.7	+10.4	+0.06	+0.04	+0.47
31				+	−4			23	15	135	+235	+1.2		—	~	~		+0.30	+0.[illegible]
32		+			+		—		3	100	+125			+ 80	−0.3	+ 1.3	—	−0.63	+0.03
33						3	—	13	+ 1	+335	~			− 75	1.6	29.7	0.0[illegible]	+0.0[illegible]	+1.[illegible]
34					2			20		+135		+0.7	—	− 30	1.5	11.5	+0.0[illegible]	+0.51	+1.0[illegible]
35				+	−6			12		385	+140	+2.7	2.5	380	−0	13.0	−0.0[illegible]	+0.67	+1.2[illegible]
36							+	30	25	+ 85	+190	+0.3	+0	− 30	−1.6	−22.5	+0.10	+0.28	+0.0[illegible]
37								28	+ 3			+1.5		− 15		—	−0.01	+0.30	+0.[illegible]
38					1	+4			—	+130	75	1.5	+1.6	+ 35	−0.6	+ 2.5	−0.10	−0.25	0
39							+40	—	+ 2			−0	—	+215	1.1	− 7.1	0.0[illegible]	−0.44	−0.70
42	f				+4		—		+	+ 80	− 30			−155	−3.0	−18.[illegible]	0.15	+0.13	+0.57
43								30		+ 15	175		—	− 85	−1.6	+ 7.5	+0.17	−0.0[illegible]	+0.5[illegible]
44								30	—	+135			+0.5	+ 90	3.3	−10.7		+0.30	+0.01
45							30	6	24	+385	335	3	—	330	−0.4		0.[illegible]	+0.27	+1.0[illegible]
46					—		—			0	385	—	—		−1.7	—		−0.17	+0.01
47										− 65	85	—		75		− 3.[illegible]	0.[illegible]	+0.1[illegible]	+0.[illegible]
48								+ 1	+	+400	+ 75		+3.5	—	−3.3	27.1		−0.3[illegible]	+0.[illegible]
49									—	+325	300	−0.7	0.8				+0.[illegible]	+0.1	+0.[illegible]
50							+30	0	+25	85	385	2.6	—	+ 80	1	+ 1.0	−0.07	+0.23	+0.[illegible]
[illegible]					—				—	85	+385	2.1	+2.4	−130	1	− 0.[illegible]	+0.02	+0.15	+0.[illegible]
52							6		− 1	35	120	+0.7	1.0	− 8	−0.9	+ 6.1	−0.05	−0.15	+0.57
53								−60		105	−130		—	+290	−2.4	13.[illegible]	0.24	−0.[illegible]	−0.4[illegible]
54							36				125				—	10.[illegible]	—	+0.3[illegible]	+0.7[illegible]
55								36	17	130	365		—	—	+	[illegible]	—	+0.3[illegible]	+0.2[illegible]
56								−40			—		—	380	+3.5	21.1	—	−0.4[illegible]	−0.7[illegible]
[illegible]								30		60	385	2.8	+4	− 25			−0.08	−0.2[illegible]	−0.[illegible]
58								27	38	300				30	.2	+	+0.5[illegible]	−0.[illegible]	−0.[illegible]
59							+45			380	300	+4.0	+4	85	+0.5	27.[illegible]	0.01	−0.6[illegible]	−0.[illegible]
60									+47	385	30		—	−380	−0		+0.0[illegible]	−0.[illegible]	−0.[illegible]
[illegible]						+4		+	+	270	135	2.2	1	+ 30	—	—	+0.01	−0.[illegible]	+0.[illegible]
62								7	+4	− 65			—			—			
				+				20	+	300	—	1.2	—	100	—	—	0.14	−0.[illegible]	−0.3[illegible]

during heart catheterization

Table continued

	Preoperative catheterization at rest				Preoperative catheterization during exercise														
Case no	Mean pressure in systemic artery mm Hg	Pulmonary vascular resistance "units"	Peripheral vascular resistance, "units"	Specific ventilation, ventilation, ml BTPS/min / oxygen uptake ml STPD	Oxygen uptake ml STPD/min	A–V oxygen uptake ml/l	Cardiac output, l/min	Stroke volume, ml	Pulmonary artery mean pressure, mm Hg	Pulmonary arterial wedge mean pressure mm Hg	Mean pressure in systemic artery mm Hg	Pulmonary vascular resistance "units"	Peripheral vascular resistance, "units"	Load, kpm/min	Ventilation, l BTPS/min	Respiratory quotient	Valvular and mural lesion before operation	Degree of mitral stenosis before operation	[illegible]
1	92	0.33	15.6	0.028	703	80.5	8.7	73	39	47	106	1.38	12.2	200	23.1	0.94	A	1	
2	84	0.43	12.8	0.033	976	94.1	10.4	95	43	35		0.77		400	22.6	0.84	A	2	
4	91	2.11	14.9	0.035	546	83.8	6.5	45	65	50	95	2.31	14.6	100	17.7	0.72	A	1	
5	102	1.85	18.0	0.040	792	90.9	8.7	104	52	40	101	1.38	11.7	200	20.3	0.85	A	2	
6	89	1.51	13.3	0.028	1 118	93.7	11.9	78	62	37	115	2.10	8.7	400		0.94	A	1	
7	93	1.28	12.3	0.023	746	83.0	9.0	90	52	41	104	1.27	11.6	150	19.9	0.73	A	1	
8	72	1.29	7.4	0.037	820	65.5	12.6	88	61	37	91	1.80	7.2	200	27.7	0.82	A	2	
9	86	1.22	16.9	0.041	801	108.8	7.5	54	50	37	83	1.48	11.6	300	22.8	0.74	A	1	
10	82	1.73	15.0	0.039	580	87.3	6.6	45	48	35	87	1.67	13.2	100	19.1	0.81	A	1	
11	108	0.24	25.2	0.032	350	86.7	6.5	—	43	39	120	0.64	19.0	100	15.4	0.78	A	1	
12	89	1.36	10.9	0.036	—	—											B	2	1
13	90	4.00	18.2	0.029	—	—											A	1	1
14	92	1.74	18.9	0.033	634	87.2	7.3		37	4.	119	2.05	16.5	100	21.6	0.82	A	1	1
15	78	0.80	6.5	0.024	1 125	99.8	11.3	87	43	39	90	0.40	8.0	400	33.1	0.97	A	2	1
16	84	2.50	12.1	0.032			—	—		~							B	1	1
17	86	0.91	12.4	0.031		—	—										B	2	1
18	78	7.87	11.5	0.040	870	121.2	7.2	53	97	55	87	0.61	12.1	200	35.5	0.85	B	1	1
19	119	4.78	24.0	0.026	920	140.8	6.5	—	77	55	108	2.70	18.2	300	30.8	1.05	A	2	1
20	83	0.20	15.7	0.033	667	86.9	7.7	55	33	33	92		11.9	150	18.6	0.78	A	2	1
21	102	1.90	24.3	0.037	600	101.1	6.0		44	34	119	1.67	18.3	75	18.1	0.80	B	1	1
22	87	1.96	16.5	0.029	614	90.4	6.8		40	31	104	2.50	15.3	100	18.3	0.94	A	2	1
23	92	2.93	19.3	0.034		—											B	1	1
24	87	2.73	19.8	0.036	725	92.7	7.8	57	72	42	93	3.64	11.0	200	27.7	0.93	A	1	1
25	117	5.00	22.1	0.029													B	1	1
26	78	2.55	15.5	0.035	507	68.4	7.4	55	63		100		13.5	75	17.0	0.83	A	1	1
27	103	0.77	18.8	0.040													B	1	L
28	90	5.98	19.5	0.053													A	1	L
29	87	1.47	12.4	0.031	741	80.3	9.2	68	53	34	107	2.07	11.6	200	21.2	0.84	A	1	L
30	80	1.53	10.7	0.024	822	86.4	9.5	98	61	44	90	1.79	9.5	200	19.3	0.85	B	1	L
31	82	10.00	13.4	0.045													A	1	B
32	95	2.14	16.8	0.037	901	118.7	7.6		68	41	107	3.55	14.1	300	24.2	0.89	A	1	L
33	80	2.44	16.9	0.033	931	134.9	7.0		55	35	98	2.86	13.7	300	38.5	0.93	B	1	S
34	78	0.88	10.7	0.034	1 072	107.8	9.9	72	61	30	105	1.11	10.6	400	35.5	1.03	A		B
36	80	10.00	25.9	0.040	369	123.6	2.9	21	98		89		30.7	75	23.6	1.01	B		L
37	83	1.41	12.8	0.039													A	1	L
38	81	1.07	9.9	0.024													B	1	S
39	78		18.3	0.034	516	82.5	6.3	43	80	44	100	2.54	15.9	100	18.1	0.89	B	1	S
40	80	0.95	10.0	0.026	1 206	104.2	11.6	87	61		105		9.1	400	29.0	0.90	B	2	S
41	77	2.26	24.2	0.032													B	1	S
42	77	0.45	25.3	0.035													A	1	L
43	87	0.87	14.4	0.029	765	106.3	7.2	45	65	49	92	1.30	12.8	300		0.85	B	1	L
44	75	4.41	11.0	0.030													B	1	S
45	75	0.89	21.5	0.025													B	1	
46	93	2.42	26.4	0.035	695	122.0	5.7		54	40	107	2.46	18.8	150	23.6	0.91	B	2	S
47	79	2.17	12.0	0.032	762	92.8	8.2	71	34	34	93	2.44	11.3	150		0.97	A		S
48	90	0.93	11.7	0.028	1 212	90.2	12.2	82	31	24	104	0.34	8.5	400	37.8	0.83	A		S
49	74	0.75	10.9	0.038	591	74.2	8.2	62	43	30	81	1.58	8.9	100		0.84	A	2	L
50	77	1.86	15.9	0.031													A	1	L
51	72	1.00	11.5	0.026	632	87.8	7.2	70	43	33	86	1.38	11.9	200	16.3	0.83	B	1	L
52	81	2.05	17.7	0.027	632	94.3	6.7		30	24	91	1.79	13.6	200	18.5	0.97	B	2	S
53	77	12.69	27.7	0.037													A	1	B
54	68	0.81	11.6	0.036	824	97.6	8.4	63	33	37	81	1.90	9.8	200	26.2	0.96	B	1	S
55	79	14.24	16.7	0.037													B	1	L
56	81	0.39	10.4	0.028	949	93.8	10.1	79	43	30	89	1.29	8.8	300	23.4	0.90	B	2	S
57	130	1.27	20.5	0.028	994	114.7	8.7		5	43	44	0.92	18.8	300		0.88	B	1	S
58	77	3.33	15.4	0.036	628	72.8	8.6	70	71	46	79	2.9	9.2	100		0.50	A	1	S
59	75	5.43	20.5	0.029	953	123.8	7.5	32	83	46	98	3.20	13.1	200	39.2	1.22	B	2	B
60	76	2.17	15.7	0.034													B	1	S
62	91	3.00	27.0	0.040	549	80.7	6.8	60	39	41	98	2.63	14.4	100	13.9	0.83	B	2	B
72	91	2.15	17.2	0.027													A	1	S

Table continued

Case no.	[illegible]
Heart rhythm at ... surgery / ... investigation	[illegible]
Post-operative changes	
Appearance of ...	[illegible]
Appearance of ...	[illegible]
Change of ...	[illegible]
Q ... interval, ...	[illegible]
... interval, ...	[illegible]
... in frontal plane degrees	[illegible]
QRS ... in frontal plane degrees	[illegible]
Net area ...	[illegible]
...	[illegible]
...	[illegible]
W ...	[illegible]
W ...	[illegible]
...	[illegible]
...	[illegible]
...	[illegible]
...	[illegible]
...	[illegible]
...	[illegible]

Table continued

	Post-preoperative changes				Postoperative values				Post-preoperative changes at rest								
Case no	Vital capacity (VC) l BTPS	Total capacity (TC) l BTPS	FRC/TC %	RV/TC %	Arterial oxygen saturation, % at rest	Oxygen uptake ml STPD/min at rest	A–V oxygen difference ml/l at rest	Cardiac output, l/min at rest	Stroke volume ml	Stroke volume/Blood volume ml/l	Mean pressure in right atrium, mm Hg	End-diastolic pressure in right ventricle mm Hg	Pulmonary artery mean pressure mm Hg	Pulmonary arterial wedge mean pressure mm Hg	Mean pressure in brachial artery mm Hg	Right ventricle work against pressure mm H	Pulmonary vascular resistance units
1	−0.36	+0.01	+ 2	+10	96.3	237	61.7	3.8	—	—	−3	+3	—	—	+18	−0.78	—
2	−0.89	−0.01	+ 8	+12	96.0	266	44.2	6.0	− 2	+0.3	+2	+5	− 3	− 6	−10	−1.08	+0.
4	+0.16	+0.22	+10	+ 1	96.6	193	52.3	3.7	—	—	—	—	—	—	+ 3	—	
5	−0.92	−0.89	+ 3	− 2	94.4	281	48.8	5.8	—	—	−4	0	—	—	−14	−0.31	
6	+0.08	+0.43	+12	+ 5	96.8	209	37.6	5.6	−11	+0.1	+1	+1	−12	− 1	−13	−1.64	−0.
7	−0.53	−1.61	+29	− 7	94.7	318	56.8	3.8	—	—	−3	+1	—	—	+ 6	−2.50	
8	−0.53	+0.07	+18	+12	98.2	230	39.8	5.8	−10	+2.5	−3	−6	−22	−15	+ 3	−2.43	−0
9	−0.70	−0.28	− 3	+10	98.8	221	46.1	4.8	+10	−1.0	0	+3	− 7	− 7	+11	0.74	+0.
10	−0.09	−0.25	+11	− 3	101.2	198	43.8	4.5	+ 1	+3.3	−2	−1	−16	−14	+ 4	−1.30	−0.1
11	−0.19	−0.39	0	− 1	95.4	233	58.8	4.0	—	—	+1	+1	− 1	− 7	+10	−0.19	+1.31
12	—	—	—	—	98.5	307	50.7	6.1	−16	−0.8	−2	−5	− 4	− 5	+ 2	−0.37	+0.6
13	−0.71	−0.33	+11	+13	98.6	188	39.9	5.0	+18	+6.0	−3	−7	−23	−14	+ 4	−1.02	—
14	−0.77	−0.32	+11	+12	102.0	218	57.4	3.8	—	—	0	0	−20	−17	+ 2	−1.47	−0.
15	−0.82	−0.15	+11	+12	98.9	256	44.6	5.7	−15	−3.2	+2	+2	− 9	− 7	+21	−2.11	+0.
16	+0.03	+0.58	+ 6	+ 4	97.9	259	53.1	4.9	− 1	−2.3	+1	0	−25	−18	− 5	−2.40	−1
17	−0.90	−1.08	+ 3	+ 6	96.8	279	60.1	4.6	—	—	−2	−1	−10	−10	− 4	−1.34	
18	−0.37	−0.11	+ 8	+ 5	84.0	288	57.7	5.0	—	—	+4	−9	—	—	+ 9	−3.66	—
19	−0.47	−0.38	+ 5	+ 8	98.7	205	68.5	3.1	—	—	+5	+6	+14	+ 2	−18	−0.67	+6
20	−0.01	+0.28	+ 5	+ 5	98.7	234	78.2	3.0	—	—	+6	−2	—	—	− 8	−0.70	
21	−0.15	−0.40	+ 3	− 3	92.7	230	51.4	4.3	—	—	+4	+4	+ 3	+ 3	0	+0.18	−0
22	+0.03	−0.03	+11	− 2	97.7	239	46.8	5.1	—	—	−4	+2	—	—	− 3	0.16	
23	+0.16	+0.47	+12	+ 3	97.9	182	53.9	3.3	—	—	−9	−4	—	—	− 3	−2.03	
24	+0.11	+0.17	− 2	0	98.6	244	48.7	5.0	− 6	−1.1	0	+1	−23	−18	+18	−1.32	−1
25	−0.05	+0.13	+10	− 3	97.0	222	77.8	2.9	—	—	0	−3	− 9	—	+22	−1.52	—
26	−0.11	+0.54	+ 8	+14	96.1	208	51.8	4.0	+ 1	−0.8	+3	+3	−16	−11	− 7	−1.66	−0.5
27	−0.26	+0.18	+15	+12	89.2	215	59.5	3.6	—	—	+3	+1	− 7	−16	− 2	1.20	+2.6
28	+0.16	+0.61	+ 2	+ 4	95.3	209	64.3	3.3	—	—	−3	−9	−34	−21	− 7	−1.75	−2.2
29	—	—	—	—	97.2	239	57.3	4.2	−23	−3.9	−3	−3	−14	−10	+11	−1.28	−0
30	+0.26	+0.30	+ 5	− 1	94.3	247	48.9	5.1	−11	−3.7	0	0	− 4	− 2	+ 3	−1.46	+0.2
31	+0.65	+1.04	+ 6	1	98.0	181	54.2	3.6	− 1	—	−6	−4	−43	−23	+ 7	4.70	−7.7
32	−0.12	−0.77	+ 7	− 6	97.6	168	60.0	3.1	—	—	−1	−7	− 9	− 7	− 5	−0.89	+1
33	+0.19	+0.22	+ 5	− 1	97.7	268	61.8	4.4	—	—	−3	−3	−15	−11	+ 5	0.81	0.
34	+0.03	+0.57	+13	+ 6	98.3	241	47.4	5.1	−12	−0.3	−3	+3	−10	− 9	+ 5	−1.49	0.1
36	+0.71	+1.31	+ 7	+ 4	98.3	249	59.7	4.1	+22	+6.0	−2	−2	−20	−12	—	0.16	−4
37	+0.20	+0.46	+11	+ 4	98.8	254	41.2	6.2	+18	+7.0	+2	−2	−29	−24	− 2	−2.67	0.7
38	+0.16	+0.66	+ 7	+ 5	—	—	—	—	—	—	—	—	—	—	—	—	
39	+0.12	−0.35	+ 3	− 1	96.8	178	46.0	3.8	+ 1	+0.6	−3	+1	−18	—	− 8	1.17	
40	−0.86	−1.30	+ 6	− 2	97.8	246	42.5	3.8	−18	−2.6	−6	−3	− 3	− 4	− 2	−0.40	+0.
41	+0.08	+0.21	+ 4	+ 1	97.9	268	70.4	3.8	—	—	0	+3	−17	−13	0	0.78	1
42	+0.10	+0.02	+ 5	− 2	95.4	209	73.5	4.1	—	—	−1	−5	−13	− 8	− 2	−0.02	2
43	−0.34	−0.08	+ 1	+ 7	—	—	—	—	—	—	—	—	—	—	—	—	
44	+1.68	+1.85	+ 3	− 6	97.1	235	50.6	4.6	−11	0	−3	−3	−40	−19	− 6	1.90	3
45	−0.48	−0.65	+ 8	+ 1	98.4	227	46.8	4.8	—	—	+1	+1	—	—	8	0	
46	−0.53	−0.21	+10	+ 8	97.5	190	59.4	3.2	—	—	−4	−6	−14	−11	−19	0.38	0
47	+0.41	+0.02	− 6	− 8	—	—	—	—	—	—		—	—	—			
48	+0.18	+0.29	+12	0	95.6	209	47.0	5.7	−21	—	+2	—	0	+ 1	−12	0.50	8
49	+0.22	+0.47	0	+ 1	—	—	—	—	—		+3	—	—	—	—	—	
50	+0.33	+0.38	+ 4	0	98.2	243	58.2	4.2	−11	+1.0	−2	+3	−18	− 8	+ 8	−1.35	0.2
51	−0.17	−0.22	+ 9	− 1	95.4	256	50.5	5.1	−17	+4.3	+2	+6	+11	+ 8	− 1	0.13	0.5
52	−0.40	−0.55	− 3	− 1	97.7	230	49.2	4.7	—		0	+1	0	—	+ 6	0.01	0.5
53	+0.54	+1.15	+ 3	+ 5	—	—	—	—	—			—	—	—	—		
54	+0.50	+0.88	− 4	+ 3	97.8	244	57.7	4.2	—		+1	4	—	—	+ 8	0.77	
55	−0.33	−0.85	− 4	0	99.0	261	60.1	4.3	—	—	−8	9	−23	+ 8	+14	−0.58	10.5
56	−0.71	−0.99	− 3	0	98.3	203	42.1	6.3	−19	—	−3	−3	− 6	−10	19	0.63	0.7
57	−0.40	−2.37	+ 5	+ 2	95.3	264	31.4	5.1	—	—	0	3	+ 3	+ 3	−25	−0.07	0.6
58	+0.21	+0.17	− 7	− 2	—	—	—	—	—	—	—	—	—	—	—	—	
59	−0.64	−1.16	+ 1	− 1	94.1	233	77.4	3.0	—	—	+2	1	+10	+14	+28	0.23	0.4
60	+0.12	+0.07	+ 9	− 2	96.4	290	44.5	6.5	+12	—	−4	+2	−12	− 9	+15	−0.61	1.0
62	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
72	−0.16	−0.26	− 6	− 1	94.4	268	34.1	7.7	28	8.7	+5	+4	−11	−11	− 6	−0.61	0.0

Table continued

Case no.	Post-operative changes: peripheral vascular resistance at rest	Post-operative changes: specific ventilation, ventilation ml STPD/oxygen uptake ml STPD at rest	Index of ... at rest	Load, kpm/min	Oxygen uptake, ml STPD/min	A-V oxygen difference ml/l	Cardiac output, l/min	Stroke volume, ml	Pulmonary artery mean pressure, mm Hg	Pulmonary arterial wedge mean pressure, mm Hg	Ventilation, l BTPS/min	Respiratory quotient	Mean pressure in systemic artery, mm Hg
		0	45 0	200	885	112.0	8 0	47	43	27	18.5	0.83	108
		—	—	400	1 16	99.8	11.2	118	34	22	23.9	0.83	—
	0 1	−0 007		100	557	99.5	5	48	28	16	14 6	0.79	85
	—	−0 007	84 7	300	878	90 6	9 1	85	52	36	31.5	0.83	96
5		−0 004	88 3	400	1 045	83.5	12.5	88	36	32	—	0.94	94
	1.5		88	300	1 018	117.1	8.7	11	34	7	26.2	0.79	133
		−0 008		200	671	88	10.1	87	37	20	21.8	75	98
	0 1	−0 003	103	300	834	90 6	9.2	73	32	22	24.7	0 90	112
9	2.7	−0 012	94	100	531	70.2	7.1	85	27	7	1	0.87	87
10	3 7	−0 007	61.8										
	1.6	001		75	603	91.8	6 6	56	52	34	17 8	0.87	103
11	+ 4.5	−0 004	39					63	55	41	20.1		11
12	0 4	−0 008	43	200	76	92 3	8.8	40	38	25	19	89	141
13	4 5		7.8	100	887	108.5	8.3				—		95
14	0	−0.003	35 2	400	1 089	105	10.	71	4	34	29 2	91	91
15	3 4	−0 017	71.	300	1 293	1 3.5	11	82	4	36	28.8		—
16	6	−0 002										90	101
17		007	34	200	735	1 1		40	74		23.5		
18		−0 009		100	812	138 9	4.6		104	29	18.8	0 84	107
19	3 7	0.000	47	130	805	120 5		42	23	16	21.1	0.80	104
20													
	2.5	−0 008	32.7	100	277	107 2	5	35	88	39	19 5	0 87	108
21		009	33	100	715	86.5	7	53	88	39	19	1	125
22		−0 01	38.1	300	821	1	5	59	24	18	2	1.01	102
23	2	−0 000	83	300	757	95 9	7.9	88	21	10	16.8	0 83	108
25	25 1	002		200	740	9	8.3	66	85	—	21 9	88	139
26		004	40 0	150	838	86 6	.3	67	85	33	18.8	93	101
27			29	200	730	139	5.2	36	32	38	15 6	89	128
28				100	5	100	5.7	45	19		15	83	95
29		−0 002	83 0	100	300	85	7	83	88	43	7	0.84	95
30		003		200	771	102.8	7.5	54			14	0.81	98
31	9 6	−0 004	82 3	300	736	90 7	7 3	33	30	88	22.5	0 96	119
32		−0 003	33 6	300	908	134	7.5	78	33	—	24.5	0 97	122
33	2 2	0.000	33	300	885	108.7	8.7	30		20	37 7	96	1 1
34	3 3	007	38.0	400	76	102.7	11.5	88	46	21	44 3	98	119
35		000	34	100	654	1 3		44	82		25.8	97	105
37	5	0.014		300	882	85	8.8	64	37	25	17 4	0.85	92
38	1	−0 004	83	180	835	68.7	8.8	71	38	14	16.	0.88	83
40		001	77	400	88	100 7	12	83	36		22.1	0.85	104
42	5	000							—	—	—	—	—
44	5	−0 020	83	200	734	108.6	6	1	27	30	30 6	0 86	107
46		−0 001	31	150	630	1 .3	5.3	82	25	24	19.3	87	98
47									—				
48		−0 002	83	400	131	86 2	11.1	95	35	34	30.8	0.90	102
50		−0 004	61.7	300	770	97	7	53	30	17	21.4	90	103
51		003	72	390	88	95		87	88	42	31.7	0.88	85
52	7	0.008	80.3								—		—
53		007		250	787	90	7 8	96	15	13	17.5	0 85	81
54		0.008	49	300	86	93 6			73	23	33		116
55		007		300	856	96.8	10	98	21	13	19 2	0.71	88
56		001					—					—	
57	11.8	009							—		—		
58	1.7	004	78	400	1 727	138.3	8.8	88	49	32	30 6	0 85	91
60	4.1 3	0.5						—	—				

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SUPPLEMENTUM

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By

STEN ERIKSSON

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Studies in α_1-antitrypsin deficiency

BY

STEN ERIKSSON

LUND 1965
CARL BLOMS BOKTRYCKERI A.-B.

Translated by L. James Brown

CONTENTS

INTRODUCTION

This book is concerned with an investigation of α_1-antitrypsin deficiency with special reference to the laboratory diagnosis, heredity and clinical aspects of the condition

Chapter I

DETERMINATION OF SERUM TRYPSIN INHIBITOR CAPACITY

INTRODUCTION

Several methods for determining of serum trypsin inhibitor capacity (TIC[1]) are available. They all measure the degree of inhibition obtained when a standard amount of trypsin is added to a given volume of serum A variety of substrates have been used for estimating the trypsin that is not complexed by the serum inhibitors, for example, gelatin by Hussey and Northrop (1923) casein by Grob (1943) Kunitz (1947) Peacock and Sheehy (1952) and Bundy and Mehl (1958) Hemoglobin was used by Grob (1949) and Jacobsson (1955) Unlabeled fibrin was used by Clark, Cliffton and Newton (1948) and Christensen (1949) Shulman (1952 a) introduced I^{131} fibrin *as a suitable substrate for trypsin* assay and I^{131}-casein was used by Homer et al (1960) Synthetic substrates for estimating TIC was introduced by Dyce and Haverback (1960) who used benzoyl arginine p nitroanilide (BAPNA) Homer Katchman and Zipf (1963) modified the method of Schwert and Takenaka (1955) using *benzoyl* arginine ethylester hydrochloride (BAEE) *for estimating* TIC and Schön Rässler and Alter (1962) benzoyl-d, l arginine-β-napthylamide (BAA) Extensive reviews of the subject have *been given by Jacobsson (1955) and* Homer et al (1963)

The inhibition level in human serum has usually been expressed in milligrams of trypsin inhibited per millilitre of serum The normal values obtained by various authors have varied widely but in recent years normal values of about 1 mg/ml have repeatedly been reported. Table I *gives* the normal values reported by different authors.

The reasons for this variation of normal values have been thouroghly discussed by Bundy and Mehl (1958) the source and quality of trypsin vary and therefore standardization of the trypsin used against soy bean trypsin inhibitor originally suggested by Kunitz (1947) seems essential. Most authors have since followed this suggestion.

*The stability of trypsin is of impor*tance. After Gorini (1951) and Bier and Nord (1951) had demonstrated that trypsin is unstable in the pH range of its optimal activity and that calcium ions can increase the *stability* calcium-containing buffers have as a rule been used.

---

[1] TIC denotes total serum trypsin inhibitor capacity (Homer et al. 1960)

The reversibility of the inhibition of trypsin by human serum has been studied extensively and with conflicting results. Hussey and Northrop (1923) Grob (1943) Bundy and Mehl (1958) found the inhibition to be reversible. The same result was obtained by Jacobsson (1955) using the Anson hemoglobin method for trypsin assay. On the other hand Shulman (1952 a) and McCann and Laskowski (1953) found the inhibition of trypsin by unfractionated serum to be irreversible.

This chapter is concerned with the determination of TIC by a procedure based largely on the method of Erlanger Kokowsky and Cohen (1961) for trypsin assay using benzoyl-arginine-p-nitroanilide (BAPNA). According to this procedure the p-nitro-aniline released on tryptic hydrolysis of BAPNA is measured colorimetrically. Detailed data are given on standardization, inhibition of trypsin by whole serum and normal values. BAPNA has been used for TIC estimation by Dyce and Haverback (1960) who reported a normal value of 1 15 ± 0 10 mg inhibited per ml serum. Their study included 9 normal persons (see table 1)

EXPERIMENTAL

Reagents

Trypsin Crystalline trypsin, Novo Laboratories, Copenhagen, was used throughout. That preparation contains 24 4 Anson units per gram. A working solution was prepared weekly by dissolving approximately 10 mg of trypsin in 50 ml of 0.0025 N hydrochloric acid. The solution was stored at +4 C.

Soybean trypsin inhibitor Worthington Biochem. Corp 3 times crystallized from ethanol. A working solution (stored at +4°C) was prepared weekly by dissolving approximately 10 mg in 50 ml of 0 0025 N hydrochloric acid

Substrate Benzoyl-dl, l-arginine-p-nitroanilide HCl (BAPNA) (Nutritional Biochem. Corp.) A working solution was prepared according to Haverback et al (1960) by adding 435 mg to 1 litre of distilled water which was then slowly heated to 85 C. When all the substance had been dissolved the solution was chilled in icewater. The solution was stable for at least 1 1 weeks.

Buffer 0 1 M tris buffer pH 8.2, containing 0 02 M $CaCl_2$ was used.

Acetic acid 30 in distilled water

Methods

Determination of tryptic activity The method of Erlanger et al. (1961) was adopted with minor modifications.

Increasing volumes (from 50 to 300 µl) of the trypsin working solution in test tubes were made up to 4 ml by addition of 0.1 M *tris buffer* pH 8.2. These trypsin dilution and the BAPNA solution were incubated for 15 minutes in water bath at 25°C. Then 4 ml of the BAPNA solution was added to each test tube during agitation. The enzyme substrate solution was incubated for 10 minutes at 25°C The hydrolysis was then stopped by addition of 1 ml of 30 per cent acetic acid. Blanks were prepared in the same way but without addition of trypsin. The optical density of the samples was read in Beckman B spectrophotometer at 410 mµ. The blank values were subtracted from the readings, which were then plotted against the amount of trypsin added.

Standardization of trypsin with soybean trypsin inhibitor (STI) Increasing amounts (30—300 µl of the STI working solution were diluted in test tubes with tris buffer

Chapter I

DETERMINATION OF SERUM TRYPSIN INHIBITOR CAPACITY

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The inhibition level in human serum has usually been expressed in milli*grams of trypsin inhibited per millilitre* of serum The normal values obtained by various authors have varied widely *but in recent years normal values of* about 1 mg/ml have repeatedly been reported. Table I gives the normal values reported by different authors.

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[1] TIC denotes total serum trypsin inhibitor capacity (Homer et al. 1960)

tions. This was performed by including two control samples, each of which contained 200 µl trypsin but no serum The total amount of trypsin was about 34 µg giving an optical density of about 0 400

The pH remained constant at 8.2 during the reaction.

After addition of acetic acid the pH of the reaction mixture fell to 3. No change in optical density at 410 mµ was observed on repeated determinations for 24 hours after the reaction had been stopped.

The stability of trypsin during the interval (0—15 minutes) between addition of trypsin to the tris buffer and addition of substrate was studied. No loss of tryptic activity was found to

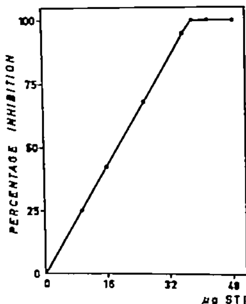


Fig. 2. Percentage inhibition of trypsin by increasing amount of soybean trypsin inhibitor (STI)

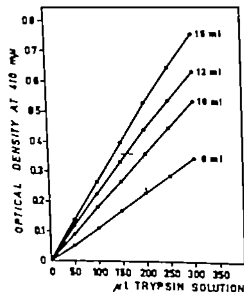


Fig. 1 Optical density t 410 mµ with varying amount of trypsin after different times of hydrolysis The broken line indicates conditions used in routine assay

occur during this interval. Bundy and Mehl (1958) found a decrease in tryptic activity despite addition of calcium to the medium. Differences in trypsin quality and composition of buffer medium might explain these conflicting findings.

It is clear from fig 2 that the inhibition of trypsin, right up to complete inhibition, varied linearly with the amount of STI added. A standardization of trypsin with STI was performed with each series of determinations. As the inhibition of trypsin by STI had been shown to be linear a single point determination (double samples) was considered sufficient.

It has repeatedly been shown that 1 mole of trypsin combines with 1 mole STI (Kunitz 1947 Sheppard and Mc

to a final volume of 3.8 ml. 200 µl of the working solution of trypsin was added to each tube and the samples placed in the water bath at 25 C for 15 minutes. Then 4 ml of substrate solution (25 C) was added. The trypsin activity was determined in the way described above. Two samples, each containing 100 µl STI were routinely included in each series of determination

Determination of total serum trypsin inhibitor capacity

a) Inhibition curves

TIC was determined in the same manner as that described for the standardization with soy bean trypsin inhibitor except than an increasing amount of serum (10—40 µl) was added. Blanks were prepared with the highest and lowest serum dilution and intermediate blank values were calculated by interpolation. Blank values were subtracted from corresponding sample values and the percentage inhibition was plotted against the amount of serum added

b) Routine assay of TIC

10 respectively 20 µl of serum were diluted with 3.8 ml 0 1 M tris buffer 200 µl of the trypsin working solution was added, and the samples were incubated at +25 C for 15 minutes. Then 4 ml substrate solution was added, and the reaction was stopped after 10 minutes by the addition of 1 ml of 30 per cent acetic acid Two control samples (C) each containing 200 µl trypsin solution but no serum, and two samples, each containing 100 µl of STI wo king solution instead f serum, were included in each series of determinations.

When the inhibition of trypsin was found to exceed 80 per cent in the 20 µl sample, the determination was repeated with 10 and 20 µl serum diluted 1 1 with saline. Only single determinations were made of each dilution. Only one blank with 20 µl serum was routinely included and the 10 µl blank value was calculated by interpolation.

Calculations

The protein concentrations (mg per ml) of the trypsin and soy bean inhibitor solutions were calculated by multiplying the optical densities at 280 mµ by the conversion factors 0.585 and 1 10 given by Kunitz (1947) The molecular weight of trypsin was taken as 23.800 (Cunningham, 1954) and that of soy bean trypsin inhibitor as 20 000 (Steiner 1954)

The following symbols were used

T —µg trypsin added.

E_s—Extinction at 410 mµ for serum sample (blank subtracted)

E_o—Extinction at 410 mµ for control (C) samples (blank subtracted)

F —Factor obtained from standardization of trypsin with STI.

V —µl serum added.

TIC (mg trypsin inhibited per ml serum—

$$\frac{E_o - E_s}{E_o} \times T \times F \times \frac{1}{V}$$

The factor F was calculated by dividing the theoretical combining ratio (µg trypsin inhibited by 1 µg STI) by the actual combining ratio found at each standardization with STI

RESULTS AND DISCUSSION

Methodological studies

An enzyme assay curve is shown in fig 1 where the optical density at 410 mµ has been plotted against amount of trypsin added. During 10 minutes hydrolysis the optical density varied linearly with the amount of trypsin added. Routine assay of TIC does not require a complete standard curve for each determination. Since minor dif ferences in optical density of the con trols occasionally occurred, a single point assay of tryptic activity was in cluded in each series of determina

of the serum to the substrate is due mainly to the opalescence, which could sometimes be detected with the naked eye. As it was found much simpler to include blanks than to centrifuge all samples in the routine assay of TIC, one blank containing 20 µl serum was routinely assayed. Higher blank values were obtained in icteric, hemolytic and lipemic sera.

The error of the method calculated on the basis of 25 double determinations (TIC range 0.28—2.40 mg trypsin inhibited per ml serum) was found to be 0.02 mg corresponding to a variation coefficient of about 2 per cent. In order to check the reproducibility two serum samples (A and B) were repeatedly determined over a period of four weeks. In these tests 1 ml of sample A was found to inhibit 0.87 ± 0.04 mg of trypsin (range 0.81—0.92). The corresponding values for sample B were 1.34 ± 0.04 with a range of 1.28—1.38. The mean values obtained in a series of 20 normal sera assayed before and after one year storage at —20 C were 0.97 respectively 0.98 mg trypsin inhibited per ml serum. Jacobsson (1955) also found TIC to be stable during storage.

Normal values

With this routine assay TIC was determined in 50 males and 50 females. The material consisted mainly of sera from registered blood donors. The female series was supplemented with 30 apparently healthy females, mainly laboratory personnel. The sera had been frozen at —20 C before analysis. The mean was found to be 1.07 and the standard deviation ±0.12. The distribution of TIC in the normal material is illustrated in fig 4.

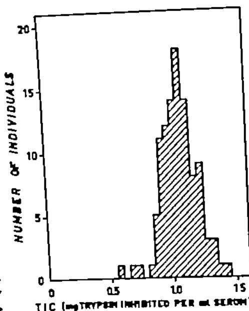


Fig. 4. Distribution of total serum trypsin inhibitor capacity (TIC) among 103 controls.

The original "normal material" consisted of 52 men (mainly registered blood donors) and 51 women (blood donors and laboratory personnel). The mean value found for the men was 1.077 and the standard deviation ±0.148 mg trypsin inhibited per ml serum. For the women the corresponding values were 1.034 ± 0.127. The difference between sexes was insignificant. Therefore both sexes were considered together. Two men had TIC values of 0.59 and 0.70 and one woman had a TIC of 0.68. The TIC values for these three individuals fell distinctly outside the others and when they were excluded from the material, the mean TIC for the remaining 100

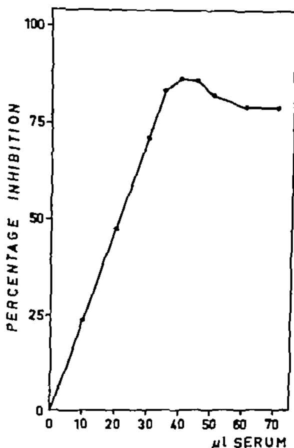


Fig 3 Percentage inhibition of trypsin by increasing amount of normal serum.

Laren 1953 Steiner 1954) The weight combining ratio originally assumed by Kunitz (1947) was 1 0 Since then various values of the molecular weights for trypsin and STI have been given (for survey see Desnuelle 1960) In the present study a theoretical weight combining ratio of 1 2 $\left(\frac{23\,800}{20\,000}\right.$ see Calculations$\left.\right)$ was used although the average ratio found was 0 94 ± 0 05 (using Kunitz original conversion factors) The factor F used in the calculations thus averaged $\frac{1\,2}{0\,94} = 1.27$ Should an other combining ratio eventually prove more correct, its application in a recalculation of the present results would offer no difficulties.

As has been pointed out by Homer et al. (1963) this standardization procedure serves to correct for differences in tryptic activity but also provides an additional check of the procedure. When the combining ratio fell outside the range 0 89—0 99 the standardization was repeated with new reagents.

In accordance with several other authors (Jacobsson 1955 Bundy and Mehl 1958 Homer et al 1963) the percentage inhibition obtained with an increasing amount of normal serum was found to vary in proportion to the amount of serum added up to about 80 per cent inhibition. The use of larger amounts of serum resulted in progressive loss of linearity and as illustrated in fig 3 an actual decrease in inhibitor capacity when more than 50 µl serum was added. The reason for this is more fully discussed in chapter IV

To be sure that the actual level of TIC fell within the linear part of the inhibition curve 10 and 20 µl serum samples were routinely examined When the inhibition obtained with the 20 µl sample exceeded 80 per cent, the assay was repeated with diluted serum Re-examination after dilution should always be done when the 10 and 20 µl values do not fall within the linear part of an inhibition curve.

The blank values obtained with 10 and 20 µl serum differed slightly but significantly from the control blanks. After centrifugation these differences diminished considerably It is therefore reasonable to assume that the increase in optical density on addition

Chapter II

ELECTROPHORETIC SEPARATION OF TRYPSIN INHIBITORS DETERMINATION OF THE α_1 INHIBITOR

INTRODUCTION

Shulman (1952b) who used a differential titration technique, was the first to produce evidence that two trypsin inhibitors exist in human serum. He also noted that one of the inhibitors had an inhibitory effect on plasmin. Jacobsson (1955) showed that the bulk, or about 90 per cent, of trypsin inhibitor capacity is found in the electrophoretic α_1 zone and the remaining 10 per cent in the α_2-zone. The α_1-inhibitor has been identified by Schultze, Heide and Haupt (1962) The purified product was an immunologically and electrophoretically homogenous glycoprotein with $S^{1.5}_{20\,W} = 3.8$. The name α_1 antitrypsin was suggested for this protein, which in addition to trypsin also inhibits chymotrypsin but not plasmin. α_1-antitrypsin is probably identical with the partially purified inhibitor described by Moll, Sunden and Brown (1958) and Bundy and Mehl (1959) The molecular weight of α_1-antitrypsin was estimated to about 60.000 by Schultze et al. (1962)

As to the nature of the α_2-inhibitor only scanty information is available. Jacobsson (1955) suggested that the α_2-trypsin and α_2-plasmin inhibitors might be identical substances. He noted that both these activities were increased in sera from patients with the nephrotic syndrome and that addition of trypsin in an amount large enough to bind all α_2-inhibitor also resulted in loss of the α_2-plasmin inhibitor capacity The absolute amount of trypsin inhibited by the α_2-inhibitor was found to be about 0.1 mg per ml serum. So far the α_2-trypsin inhibitor has not been isolated

A third trypsin inhibitor has been described by Shulman (1955) He purified a trypsin inhibitor from human serum and urine which possessed both antitryptic and antitromboplastic activity It is responsible for only a small part of the total trypsin inhibiting capacity less than 1 per cent, according to Shulman.

In order to study the partition of trypsin inhibitor capacity in different electrophoretic fractions, separation was performed in agar-gel, the sections were cut out, eluted and the trypsin inhibitor activity was subsequently determined

TABLE I *Normal values for TIC (mg trypsin inhibited per ml serum) given by different authors*

Author	Substrate	Number of normal sera analysed	TIC Mean ± S.D or range
Jacobsson (1955)	Hemoglobin	61	0.75 ± 0.11
Bundy and Mehl (1958)	Casein	40	1.03 ± 0.13
Dyce and Haverback (1960)	Benzoyl-arginine p-nitroanilide	9	1.15 ± 0.10
Homer Zipf Hieber and Katchman (1960)	I[131]-casein	103	0.87 ± 0.14
Schön, Rässler and Alter (1962)	Benzoyl-arginine-β-napthylamide	31	1.50 — 1.87
Homer Katchman and Zipf (1963)	Benzoyl-arginine-ethylester	50	0.99 ± 0.10
Present author (1965)	Benzoyl-arginine-p-nitroanilide	100	1.07 ± 0.12

individuals was found to be 1 07 and the standard deviation 0 12 mg trypsin inhibited per ml serum The three individuals with low TIC values probably represented the heterozygous state of α_1-antitrypsin deficiency which is more fully discussed in chapter V

The normal values reported in this study agree well with those obtained by other authors (see table I) The present values are directly comparable with those of Bundy and Mehl (1958) who used the same combining ratio (1.2)

The standard deviation (± 0 12) was of the same order of magnitude as that reported by other authors

SUMMARY

A simple method is described for determining serum trypsin inhibitor capacity. Benzoyl arginine nitroanilide is used as a substrate for trypsin. Under the conditions used the trypsin was stable. Standardization with soy bean trypsin inhibitor was used in each series of determinations. The inhibition produced by soy bean trypsin inhibitor was proportional to the amount added over the entire range of inhibition. The inhibition by normal serum was linear up to about 80 per cent of total trypsin inhibited The mean serum trypsin inhibitor capacity in 100 normal individuals was 1 07 ± 0 12 (S D) mg trypsin inhibited per ml serum.

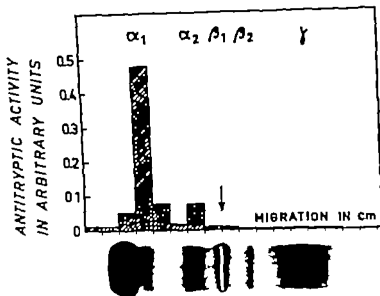


Fig. 3 Partition of trypsin inhibitor capacity in normal serum after separation in agar gel. The arrow indicates the point of pplication.

sons after the α_1-zone had been cut out *in toto* was 0 10 mg trypsin inhibited per ml serum. The standard deviation was 0 02 The value found was of the same order of magnitude as that found by Jacobsson (1955) The variation coefficient for determination of the α_2-trypsin inhibitor was as large as 15 per cent The reproducibility was low since not more than 50 µl serum could be applied to the gel without difficulty As the total trypsin inhibitor capacity of normal human serum has been shown to be 1 07 mg inhibited per ml, the average amount inhibited by the α_1 inhibitor was 0.97 mg assuming only insignificant inhibitor activity in the electrophoretic fractions outside the α_1 and α_2-zones.

SUMMARY

Separation in agar gel demonstrated two main trypsin inhibitors in human serum. In accordance with previous investigators the main activity or about 90 per cent was found in the α_1 zone. Most of the remaining activity was found in the -zone. The absolute amount of trypsin inhibited by the α_2-globulin fraction was 0 10 ± 0.02 (S.D) mg per ml serum. A small amount of inhibitor activity was found to be associated with the albumin fraction and in some sera inhibitor activity was also demonstrated in the γ zone.

EXPERIMENTAL

Material

Tris buffer 0.1 M pH 8.2, containing 0.02 M $CaCl_2$

Agar Reinagar (Behring Werke)

Methods

Agar-gel electrophoresis was run essentially in the way described by Lundh (1964) 40 µl samples of serum with an addition of brom phenol blue to stain the serum albumin were run on gel-covered glass plates 42×350 mm, in a Pherograph at 300 V potential gradient for 5 hours at +4°C. 6—8 plates were run in one chamber After electrophoresis the gels were either cut in 1 cm segments, or the separated electrophoretic fractions were cut out *in toto* and analysed for trypsin inhibitor activity For correct evaluation of the position of the different protein fractions prestained templets were used. The albumin front was marked by the bromphenol blue added. The excised gel samples were placed in weighed tubes and eluted over night at +4 C during agitation in 4 ml buffer The gel sections from two plates, corresponding to 80 µl serum, were placed in one elution tube.

Determination of trypsin inhibitor activity Suitable aliquots of the eluates, usually 3 ml, were made up to 4 ml by addition of buffer The determination was then performed in the way described in chapter I. When calculating the trypsin inhibitor capacity in the various fractions corrections for gel weights were made. All samples were centrifuged for 10 minutes at 10 000 rpm before measurement of the optical density at 410 mµ.

RESULTS AND DISCUSSION

The partition of trypsin inhibitor activity after electrophoresis in agar gel is illustrated in fig 5 As shown by Jacobsson (1955) and Dyce and Haverbach (1960) the main activity peak, comprising about 90 % of the total applied, is found in the α_1 zone and the remaining activity in the α_2-zone *The recovery in this procedure ranged* between 90 and 95 per cent In two otherwise normal sera a small activity peak was identified in the γ zone The percentage activity found in the γ zone in these sera was of the order 1—2 per cent of total trypsin inhibitor capacity When γ globulins were isolated by salt fractionation of pooled serum or by chromatography on DEAE columns and then analyzed no associated trypsin inhibitor activity could be found. Likewise, in 5 sera from patients with polyclonal hypergammaglobulinemia (2—5 g %) no activity could be demonstrated in the γ zone.

By the technique described above no inhibitor activity could be demonstrated in the albumin zone but when the albumin fraction from a large number of gels was concentrated by ultra *filtration in cellulose acetate bags* (Mies) a small amount of inhibitor activity representing about 1 % of the total inhibitor capacity was regularly found in this zone The albumin zone inhibitor was thermostable (see chapter III) and might be identical with Shulman's (1955) trypsin tromboplastin inhibitor This inhibitor has the same electrophoretic mobility as albumin

The mean value of the α_1-trypsin inhibitor determined in 10 normal per

Eberhard (1960). The α_1 and α_2-segments were cut out and the corresponding globulin fraction were eluted. The fractions were concentrated by ultrafiltration in cellulose acetate tubes (Miles) and checked by paper electrophoresis according to Laurell, Laurell and Skoog (1956). The α_1-fraction contained considerable amounts of albumin but was electrophoretically free from α_2-components.

The α_1-content of the α_2-fraction was less than 2 per cent. The trypsin inhibitor capacity of these fractions was determined according to the standard procedure described in chapter I.

Heat inactivation was performed with α_1 and α_2-fractions dissolved in phosphate buffers of varying pH and ionic strength. All experiments were carried out in a thermostat at 60°C.

Inactivation with acetone was performed at room temperature by adding 0.2 ml acetone to 0.5 ml serum sample. The mixture was agitated for about half a minute on a Vortex Jr Vibrator. After centrifugation of the mixture for 10 minutes at 4000 rpm the residual antitrypsin activity in the supernatant was determined on 100 µl samples. Blanks were included in the determination.

Inactivation with chloroform was performed as with acetone. 0.1—0.2 ml chloroform was added to 0.5 ml serum samples.

Determination of α_2-inhibitor capacity after electrophoresis in agar gel was done as described in chapter II.

RESULTS AND DISCUSSION

Fig. 6 demonstrates the inactivation of the α_1 inhibitor in 0.04 M phosphate buffer at varying pH. At pH 7.4 about 50 per cent of the initial activity is destroyed after 10 minutes at 60 C, after 20 minutes 35 per cent of the initial activity remains and after 30 minutes still about 30 per cent. Decreasing or increasing the pH of the medium results in diminished stability. To achieve complete inactivation at 60 C the medium must be kept at this temperature for 25 minutes at pH 5.1. Increasing the ionic strength of the medium results in increased thermostability. This is illustrated in fig. 7 where the remaining activity after heating the α_1 inhibitor at 60 C for 20 minutes at different pH and ionic strengths has been plotted against the pH values. In these experiments the optimal thermostability is found at pH 6.2. Corresponding experiments with the α_2-inhibitor are shown in fig. 8. At

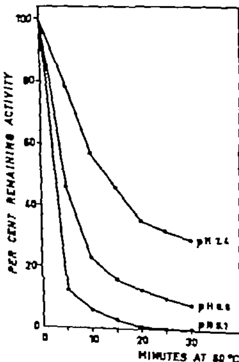


Fig. 6. The effect of pH and duration of heating at 60°C on the stability of the α_1-inhibitor. 0.04 M phosphate buffer

STABILITY OF TRYPSIN INHIBITORS DETERMINATION OF THE α_2 INHIBITOR AFTER SELECTIVE INACTIVATION OF THE α_1 INHIBITOR

INTRODUCTION

Shulman (1952 b) studied the heat inactivation of trypsin and plasmin inhibitors of undiluted serum at 60°C. He *found that the inhibition of trypsin* (and chymotrypsin) decreased to 10 per cent of the original value in approximately 20 minutes at 60°C while the inhibition of plasmin remained practically unchanged. More than 60 per cent of the inhibition of plasmin was still present after 2 hours at 60°C. His experiments demonstrated the lability of the trypsin inhibitor compared with that of the plasmin inhibitor in whole serum.

The lability of the α_1 inhibitor at elevated temperature and at an acid pH has been studied by Moll et al. (1958) Bundy and Mehl (1959) and Schultze et al. (1962) The last mentioned authors found a rapid inactivation of the α_1 *inhibitor at pH 4.5 and at 56°C.* At pH 7 0 the activity decreased to about 50 per cent when heated at 56 C for 30 minutes and after further 30 minutes about 50 per cent of the remaining activity disappeared. No systematic studies on the thermolability of the α_1 inhibitor at different pH values *and varying ionic strength seem* to have been reported

Christensen (1946) when studying the activation of plasminogen by chloroform, noted a rapid drop in the level of protease inhibitor capacity when serum was shaken with 10 per cent chloroform. Astrup (1956) found acetone to be more effective than chloroform in the removal of the trypsin inhibitor from serum. Jacobsson (1955) noted an *inactivation of the* α_1 inhibitor when stored with ether in the cold.

The present chapter gives results on the stability of α_1 and α_2-trypsin inhibitors under varying conditions. The aim was to find suitable conditions for selective inactivation of the α_1 inhibitor *in order to allow estimation of the* α_2-inhibitor capacity Such a procedure would make a preliminary electrophoretic separation of the inhibitors unnecessary

EXPERIMENTAL

Material

Phosphate buffers 0 04 M—1 0 M pH 5.1—9.5.
Acetone p.a. Merck.
Chloroform p.a. Merck.

Methods

Separation of α_1 and α_2-trypsin inhibitors
Pooled sera were fractionated by electrophoresis on pevicon blocks according to Müller

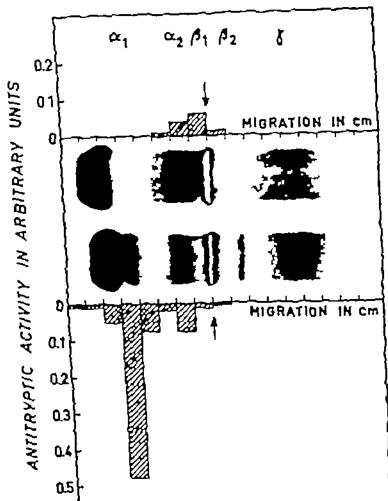


Fig 11 Partition of trypsin inhibitor capacity in agar gel after treatment of normal serum with 40 per cent acetone at pH 7.0 (above) compared with untreated normal serum (below) Arrows indicate points of application.

was achieved with 20 per cent acetone. Increasing the pH resulted in increased stability with an optimum at pH 7.0. Addition of 40 per cent acetone was found necessary for complete inactivation at pH 7. Fig 11 shows the agar gel electrophoretic fractionation of whole serum after treatment with 40 per cent acetone. The α_1-globulin band and the antitryptic activity in this region disappears completely while the antitryptic activity in the α_2-region seems to remain unchanged. The other electrophoretic serum protein fractions also appeared unchanged with the exception of the β_1C-glo-

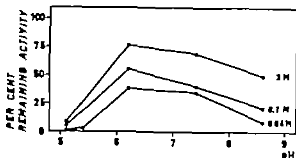


Fig 7 The effect of ionic strength and pH on the stability of the α_1 inhibitor by heating at 60°C for 20 minutes.

pH 5 1 there was a rapid initial drop in activity to about 50 per cent, but prolonged heating resulted in only a small further loss of activity Whether the two parts of the inhibition curve

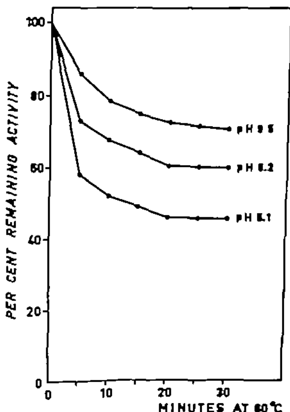


Fig 8. The effect of pH and time of heating at 60°C on the stability of the α_1-inhibitor 0.04 M phosphate buffer

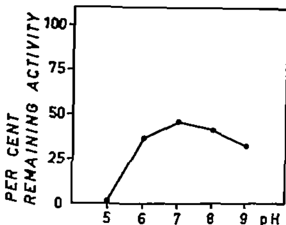


Fig 9 The effect of 20 per cent acetone on the stability of the α_1 inhibitor at varying pH.

represent two α_1-trypsin inhibitors of different thermo-stability is not known. Increasing the pH of the medium resulted in increased thermal stability of the α_1-inhibitor Despite the use of different pH levels and duration of heating no suitable conditions for the selective inactivation of the α_1 inhibitor could be found.

The inactivation of the α_1 inhibitor with acetone is illustrated in figs. 9 and 10 The reaction was pH-dependent At pH 5 complete inactivation

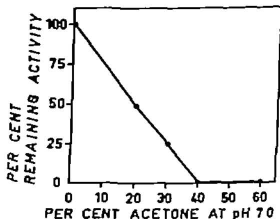


Fig. 10 The effect of increasing amount of aceton on the stability of the α_1 inhibitor at pH 7.0

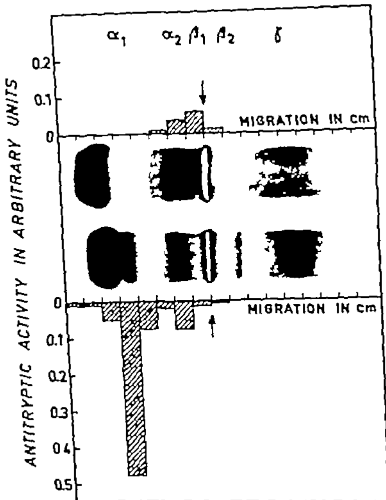


Fig 11 Partition of trypsin inhibitor capacity in agar gel after treatment of normal serum with 40 per cent acetone at pH 7.0 (above) compared with untreated normal serum (below) Arrows indicate points of application.

was achieved with 20 per cent acetone. Increasing the pH resulted in increased stability with an optimum at pH 7.0 Addition of 40 per cent acetone was found necessary for complete inactivation at pH 7 Fig. 11 shows the agar gel electrophoretic fractionation of whole serum after treatment with 40 per cent acetone. The α_1-globulin band and the antitryptic activity in this region disappears completely while the antitryptic activity in the α_2-region seems to remain unchanged. The other electrophoretic serum protein fractions also appeared unchanged with the exception of the β_1C-glo-

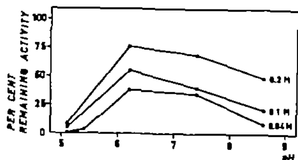


Fig 7 The effect of ionic strength and pH on the stability of the α_1 inhibitor by heating at 60 C for 20 minutes.

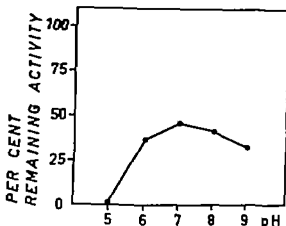


Fig 9 The effect of 20 per cent acetone on the stability of the α_1 inhibitor at varying pH.

pH 5 1 there was a rapid initial drop in activity to about 50 per cent but prolonged heating resulted in only a small further loss of activity Whether the two parts of the inhibition curve represent two α_1-trypsin inhibitors of *different thermo-stability is not* known. Increasing the pH of the medium resulted in increased thermal stability of the α_1-inhibitor Despite the use of different pH levels and duration of heating no suitable conditions for the selective inactivation of the α_1 inhibitor could be found

The inactivation of the α_1 inhibitor with acetone is illustrated in figs. 9 and 10 The reaction was pH-dependent. At pH 5 complete inactivation

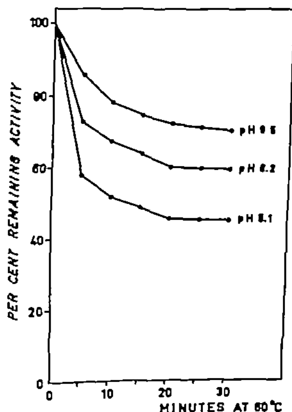


Fig 8. The effect of pH and time of heating at 60°C on the stability of the α_1-inhibitor 0.04 M phosphat buffer

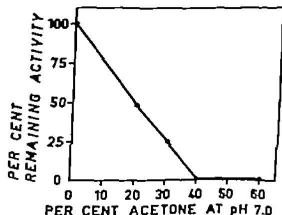


Fig 10. The effect of increasing amount of acetone on the stability of the α_1-inhibitor at pH 7.0.

with only a slight simultaneous inacti vation of the α-inhibitor and thereby allowed a rough estimation of this in hibitor without electrophoretic separa tion of the trypsin inhibitors. The mean value of the α_1-inhibitor after acetone treatment wa 0 13±0.02 (S D) mg trypsin inhibited per ml serum

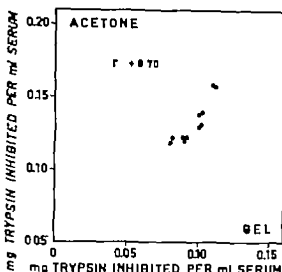


Fig 12 Mg trypsin inhibited per ml serum by the α_2-inhibitor Values obtained in 23 normal sera after treatment with acetone plotted against values obtained after electrophoresis in agar gel.

bulin which disappeared Whether this depended upon a transformation into the β_1A globulin or denaturation has not been investigated. The inactivation of the α_1 inhibitor was found to be momentary and there were no signs of reversibility of the inactivation. After addition of isolated α_1 globulin fraction to whole serum with a consequent final antitryptic activity of 3—4 times the original activity complete inactivation was also achieved by addition of 40 per cent acetone. It was found unnecessary to correct the pH of serum with buffer before addition of acetone most sera having a pH of 7.5—8 5 The stability of the α_2-inhibitor under the same conditions (40 per cent acetone, pH 7 0) tended to vary the mean residual activity being about 80 per cent In some experiments not more than 65 per cent of α_2-inhibitor activity persisted after acetone treatment In spite of this variable stability of α_2-inhibitor a fairly good correlation was found between values obtained with acetone and those noted on elution from gels. This is illustrated in fig 12 The correlation coefficient was +0 70 The mean value for the α_2-inhibitor as determined after acetone treatment was somewhat higher than the corresponding value obtained after elution from gels, 0 13 ± 0 02 (S D) mg as compared to 0 10 mg trypsin inhibited per ml serum. This difference might in part be due to the occurrence of small amounts of acetone-stable trypsin inhibitors outside the α_1 and α_2-regions (see chapter *II*)

When chloroform was used for inactivation, the results resembled those obtained with acetone, but no proportionality was found between the remaining inhibitor activity and amount of chloroform treated serum

SUMMARY

The stability of the α_1 and α_2-trypsin inhibitors was studied under various conditions The thermolability of the α_1 inhibitor compared with that of the α_2-inhibitor found by other investigators was confirmed The influence of pH and ionic strength during heat denaturation was studied Treatment of serum with 40 per cent acetone completely inactivated the α_1 inhibitor

with only a slight simultaneous inactivation of the α_1-inhibitor and thereby allowed a rough estimation of this inhibitor without electrophoretic separation of the trypsin inhibitors. The mean value of the α_1-inhibitor after acetone treatment was 0 13 ± 0 02 (S D) mg trypsin inhibited per ml serum

Chapter IV

RECOGNITION OF α_1 ANTITRYPSIN DEFICIENCY

INTRODUCTION

The fairly well outlined band normally occurring in the slow α_1 zone on paper electrophoretic strips is produced by the α_1 antitrypsin. A series of 5 patients was described where the serum analysis revealed no α_1 band in the electrophoretic strips and only traces of α_1 antitrypsin with slightly reduced mobility on immunoelectrophoresis in agar gel but no other abnormalities in the serum proteins. To describe the condition, which fulfilled these three criteria, the name α_1 antitrypsin deficiency was introduced (Laurell and Eriksson, 1963). This chapter gives a survey of electrophoretic findings in different media and data on the total trypsin inhibitor capacity (TIC) in α_1 antitrypsin deficiency. Determinations of non-α_1 antitrypsins in this condition are also given.

EXPERIMENTAL

Material

Sera from 38 individuals (20 were males and 18 females) in which the above-mentioned three criteria of α_1 antitrypsin deficiency were fulfilled have been studied. All sera had been frozen at —20°C.

Methods

Paper electrophoretic analysis was performed according to Laurell, Laurell and Skoog (1956). Normal value for α_1 globulins 0.23—0.38 g %.

Agar gel electrophoresis with subsequent determination of antitryptic activity as described in chapter II.

Immunoelectrophoresis was run on microscopic slides according to Heremans (1960) modification of Scheidegger's technique.

α_1-antitrypsin antiserum was prepared in accordance with Laurell and Eriksson (1965).

Determination of total trypsin inhibitor capacity was performed as described in chapter I with minor modifications.

Determination of α_2-trypsin inhibitor capacity was performed after acetone treatment (chapter III).

α_1 and α_2-globulin fractions were prepared in the way described in chapter III.

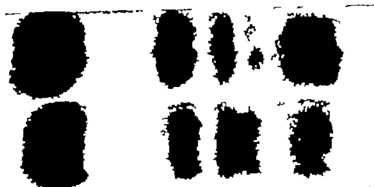


Fig 13. Paper electrophoretic pattern of serum in α_1-antitrypsin deficiency (above) compared with that of normal serum (below)

RESULTS AND DISCUSSION

The electrophoretic pattern in α_1-antitrypsin deficiency

The pattern obtained on paper electrophoresis is illustrated in fig. 13. The *normally well demarcated α_1 band is* missing, but the zone between albumin and the α_2-zone otherwise appears normally stained. It has also been shown (Laurell and Eriksson, 1963) that the α_1 lipoprotein and the orosomucoid, which constitute most of the remaining components of the α zone, are normal in α-antitrypsin deficiency. In fig. 14 the numerical value of the α_1-globulin fraction after paper electrophoresis of sera from 35 individuals with α antitrypsin deficiency have been plotted against the trypsin inhibitor capacity (TIC). It is clear that no correlation exists between these two parameters. Most of the deficiency sera show subnormal α_1 values, but several show α-globulin values falling within the normal range (0.23—0.38 g %). Therefore, a normal α_1-globulin values does not exclude the presence of α-antitrypsin deficiency.

Fig. 15 shows the protein pattern and distribution of antitryptic activity in serum, after electrophoresis in agar gel, from an individual with α_1-antitrypsin deficiency and, for comparison, of a normal serum. The reduction in antitryptic activity in the α_1-region is apparent. As in paper electrophores-

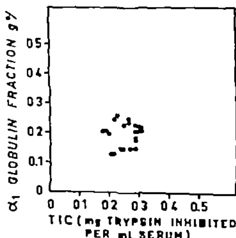


Fig. 14. Total serum trypsin inhibitor capacity (TIC) in 35 sera with α_1-antitrypsin deficiency plotted against electrophoretic α_1-globulin values.

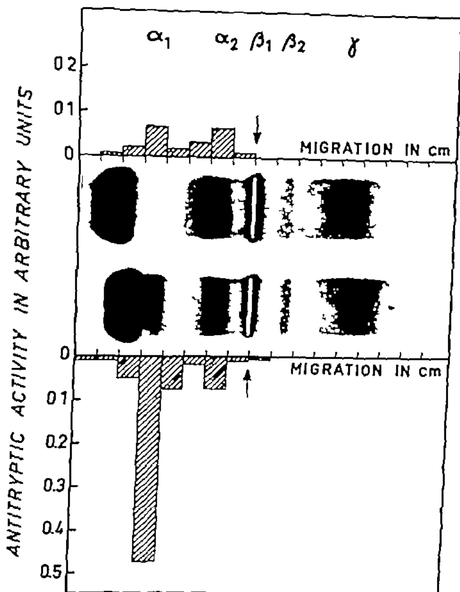


Fig 15. Partition of trypsin inhibit r capacity after separation in agar gel. Serum from an individual with α_1-antitrypsin deficiency (above) compared with a normal serum (below)

is, the sharply demarcated α_1 band is *missing while the rest of the α_1 zone* seems to be normal The antitryptic activity *in the α_2-region is about the* same as in the normal serum.

The immunoelectrophoretic pattern obtained in α_1 antitrypsin deficiency is illustrated in fig 16 The pattern of normal *serum is given for comparison*

The reduced mobility *of the small amount of α_1 antitrypsin found in the deficiency serum is evident* This reduced mobility of α_1 antitrypsin on immunoelectrophoresis is a constant finding in α_1 antitrypsin deficiency and cannot be detected by ordinary electrophoresis on paper

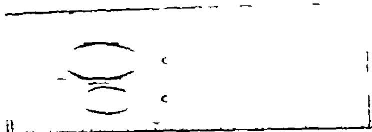


Fig. 16. Immunoelectrophoretic pattern of serum in α_1-antitrypsin deficiency (below) compared with that of normal serum (above). Antiserum specific against α_1-antitrypsin.

Trypsin inhibitor capacity (TIC) of serum in -antitrypsin deficiency

The mean value for TIC in the 38 individuals was 0.25±0.05 (S.D.) mg trypsin inhibited per ml serum, or about 23 per cent of normal (1.07). The observed range was 0.16—0.34 mg.

The α_2-inhibitor was determined in 10 deficiency sera after acetone inactivation of the α_1 inhibitor. The mean value was 0.15±0.03 (S.D.) mg trypsin inhibited per ml serum, *i.e.* a value not significantly differing from normal 0.13±0.02, given in chapter III. Jacobsson (1955) found the α_2-inhibitor to be uninfluenced in various conditions with high levels of the α_1 inhibitor. Obviously the same holds true for non-α_1 antitrypsins in the deficiency stat

The mean trypsin inhibitor capacity due to α_1 antitrypsin in the deficiency state was thus calculated as 0.10 mg per ml (0.25 minus 0.15) or about 12 per cent of the normal value. The figures given here for non-α-antitrypsins and α_1-antitrypsin in the deficiency state are in good agreement with those found by Laurell and Eriksson (1963) with an immunological method for estimation of α_1-antitrypsin.

It was pointed out in chapter I that in an investigation of normal sera for their trypsin inhibitor capacity the inhibition curve was linear up to about 80 per cent inhibition when an increasing amount of serum was added (fig. 3). In α_1-antitrypsin deficiency a largely similar curve was obtained but the loss of proportionality occurred earlier already at about 25 per cent inhibition. This is illustrated in fig. 1. Owing to the low α-inhibitor content of these sera the percentage inhibition obtained with 10 and 20 µl serum was much lower than that obtained with normal sera. To obtain accurate values of TIC in deficiency sera the determinations were made with 50 µl serum and a corresponding blank. In deficiency sera the degree of inhibition always varied linearly with the added amount of serum up to addition of 50 µl serum.

The reason why the correlation between the degree of inhibition and the amount of serum added ceased earlier when the serum was deficient in α_1 antitrypsin than when it was normal was obvious when α and α_2-globulin

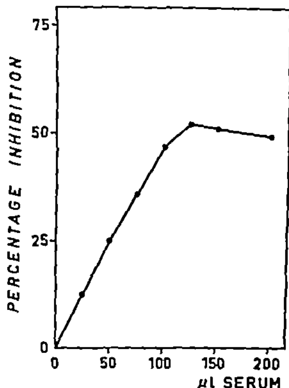


Fig 17 Percentage inhibition of trypsin with increasing amount of serum from an individual with α_1-antitrypsin deficiency

fractions were tested separately for their inhibitor capacity Fig 18 shows the inhibition curves obtained with increasing amount of these fractions added to the test system. The α_1 globulin fraction inhibited trypsin linearly over the entire range from 0 to 100 per cent, while the corresponding inhibition by the α_2-globulin fraction was linear only up to about 50 per cent inhibition. In sera with normal inhibitor capacity the shape of the inhibition curve is dictated by the large amount of α_1 inhibitor compared with that of α_2-inhibitor In α_1 antitrypsin deficiency in which α_1 antitrypsin is only about 10 per cent of normal and the activities of the α_1 and α_2-inhibitors are roughly equal, the shape of the inhibition curve is relatively more influenced by the α_2-inhibitor resulting in an earlier loss of proportionality

It is also evident from fig 18 that the inhibitor capacity of the α_2-globulin fraction decreases on successive addition of further amount of inhibiting substance. The same phenomenon was observed with both normal (fig 3) and deficiency sera (fig 17) This finding can be interpreted in the light of the demonstration by Haverbach et al (1962) that human serum contains an α_2-globulin capable of binding trypsin without inhibiting its esterolytic properties The protein responsible for this binding has recently been shown to be an α_2-macroglobulin (Mehl, O Connell and De Groot 1964) Data on the relative affinities of trypsin for

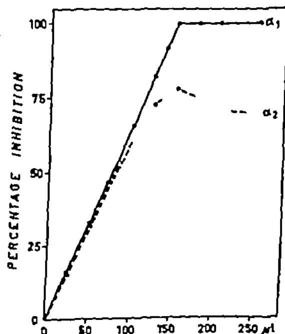


Fig 18. Percentage inhibition of trypsin with increasing amount of α_1 or α_2-globulin fractions.

the α_1-trypsin inhibitor and the α_2-macroglobulin are necessary before any valid conclusions can be drawn concerning the partition of trypsin between the two trypsinbinding α_2-globulins. Haverbach's et al. finding that about 0.1 mg of trypsin can be bound to the α_2-macroglobulin might result in erroneous determinations of total trypsin inhibitor capacity especially in α_1-antitrypsin deficiency states and in conditions associated with high levels of α_2-macroglobulin. This error may be considerable, only if the trypsin bound to the macroglobulin does not possess the same specific activity against BAPNA as unbound trypsin.

SUMMARY

The laboratory findings in sera from 38 cases of α_1-antitrypsin deficiency are described. Characteristic is the abscence of a visible band in the α_1 region in paper or agar gel electrophoresis. The immunoelectrophoretic mobility of α_1-antitrypsin was found to be reduced. The mean total trypsin inhibitor capacity was 0.25 mg trypsin inhibited per ml serum with a range of 0.15—0.34. The mean trypsin inhibitor capacity due to α_1 antitrypsin was 0.10 mg or 12 per cent of the normal level, while the corresponding capacity due to non-α_1 antitrypsins was the same as in normal sera.

Chapter V

HEREDITY IN α_1 ANTITRYPSIN DEFICIENCY

INTRODUCTION

When studying the α_1 antitrypsin activity in relatives of one patient with α_1 antitrypsin deficiency three levels of activity were found in the family members, namely a normal level, an intermediate level with about 60 per cent of normal and a markedly low level with about 10 per cent of the normal activity The observations made argued for a recessive type of inheritance for α_1 antitrypsin deficiency (Eriksson, 1964) Similar findings were made by Lopez et al. (1965) and Kueppers et al. (1965)

This chapter is concerned with the total trypsin inhibitor capacity (TIC) in sera from relatives of 14 probands with α_1 antitrypsin deficiency and the mechanism of the inheritance in this condition Data obtained in immunoelectrophoresis of sera from family members are also given

MATERIAL AND METHODS

Definitions and symbols

TIC Total serum trypsin inhibitor capacity Normal value 1 07 ± 0.12 (S.D) mg trypsin inhibited per ml serum.

In *α_1-antitrypsin deficiency* there is no distinct α_1 band on paper electrophoresis, but otherwise the serum protein pattern is normal except for occasionally increased α_2 globulin values. TIC is reduced to 0.23 ± 0 05 mg trypsin inhibited per ml serum, and there is a reduced mobility of α_1-antitrypsin on immunoelectrophoresis. (See chapter IV)

Intermediate TIC is provisionally defined as TIC between 0 4 and 0 8 mg trypsin inhibited per ml serum. (See also discussion.)

Pedigrees The same symbols as those used by Stern (1960) are employed throughout. Hatched symbols (■) indicate the presence of α_1 antitrypsin deficiency (male) half hatched *symbols* (◧) *indicate* intermediate TIC and hollow symbols (□) normal TIC. Hollow *symbols with* a broken outline ( ) denote that no determination has been performed A cross within a symbol indicates that the individual is dead. Circles (○) denotes female sex (See also chapter VII, page 44)

Figures above a pedigree refer to running number used in the clinical section (Chapter VII) and figures below an individual indicate the TIC. Complete pedigrees, when available are given in chapter VII in conjunction with the clinical studies in each family

Methods

Paper electrophoresis was run according to Laurell et al (1956) Normal value for α_2-globulins 0 43—0.05 g/100 ml serum.

Immunoelectrophoresis was run on microscopic slides utilizing Hirschfeld's (1960) modification of Scheidegger's technique

Antiserum An antiserum specific against α_1 antitrypsin was prepared as described by Laurell and Eriksson (1965)

TIC (total serum trypsin inhibitor capacity) was determined as described in chapter I Normal value 1.07 ± 0 12 (S.D) mg trypsin inhibited per ml serum

Patient material

Data useful for genetic analysis were obtained from 14 families in which a case of α_1-antitrypsin deficiency was known. During the years 1963—64 sera from 24 probands with α_1-antitrypsin deficiency were examined in the laboratory but for geographical and other reasons only 14 were suitable for family studies. TIC determinations were performed in all together 110 family members and 13 spouses of individuals with α_1-antitrypsin deficiency. 15 were children and 8 were parents of the affected individuals, 12 siblings were available for analysis. In 2 families TIC determinations were made in three generations. The control material consisted of 103 individuals and is described in chapter I.

RESULTS

Fig. 19 gives data for TIC in the whole material, except spouses, comprising 213 individuals. The scatter diagram shows three groups of individuals: probands, relatives and controls. The distribution of TIC among relatives was trimodal. One group represented the deficiency state, TIC being less than 0.4 mg/ml. One group represented the normal level with a mean TIC around 1.10 mg/ml. The majority of relatives fell between these two groups, with a mean TIC around 0.70 mg/ml. Fig. 19 contains two dividing lines, one at TIC 0.40 and one at TIC 0.80 mg. For reasons given later these two dividing points were chosen to define the lower and upper limits of intermediate TIC. In what follows, normal

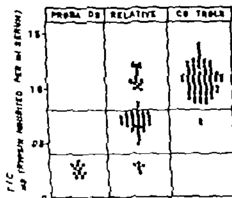


Fig. 19. TIC in probands (14), relatives (110) and controls (103).

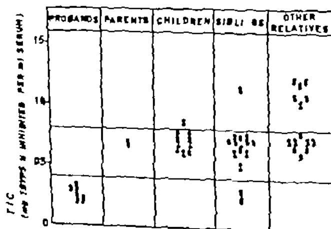


Fig. 20. TIC in probands (14), parents (8), children (15), siblings (41) and other relatives (32).

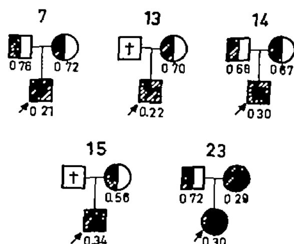


Fig. 21 Individual TIC in parents of affected individuals.

TIC thus represents values between 0 80 and 1 40 mg intermediate TIC values between 0 40 and 0 80 and low TIC values below 0 40 mg/ml.

Fig 20 shows the distribution of TIC when all relatives were divided into four subgroups namely parents, children siblings and other relatives of individuals with α_1 antitrypsin deficiency

It is clear from fig 20 that among the 8 parents studied, all but one, who satisfied the criteria for α_1 antitrypsin deficiency had intermediate TIC. It is also seen that the TIC was inter*mediate in all 15 children except 2* who had a TIC above 0 80 mg/ml Most (27) of 41 siblings had an intermediate TIC. Of the remaining siblings, roughly half fell within the normal range *and the other half* within the low range. Of other relatives, roughly half fell within the normal range and the other half within the intermediate range One individual had α_1 antitrypsin *deficiency All* siblings *and other relatives* had normal α_1-globulin values.

Three of the 103 controls had an intermediate TIC (*fig 19*)

Fig 21 gives individual TIC values found in parents. In 2 of the parents the α_1-globulin values were increased

The TIC in children of affected indi*viduals can be studied in detail* in fig 22 Fifteen children of 7 matings between one affected individual and a normal spouse were available for analysis. All spouses had a normal electrophoretic pattern. The 2 children with a TIC above 0 80 mg/ml are found in families 7 and 12. (Indicated by ques*tion marks) Both children had* increased α_1-globulin values (0 75 and 0 74 g/100 ml) In fig 23 the electrophoretic serum α_1-globulin values found in parents and children of af*fected individuals are plotted against* the TIC. A strong correlation exists be-

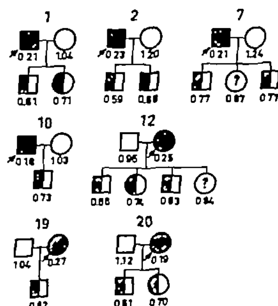


Fig 22 Individual TIC in children of affected individuals.

ween these two parameters, while no correlation exists in α_1-antitrypsin de ficiency In this condition the α_2-values were often markedly increased without any corresponding increase of TIC.

Fig 24 gives the results obtained in the analysis of the sibships. TIC deter minations were performed in 53 indi viduals belonging to 12 sibships. All three TIC ranges are represented in most of the sibships. Intermediate TIC was predominant (27 out of 53) The sex distribution of these 27 was equal (13 males and 14 females) Eighteen (12 males and 6 females) had α_1-anti trypsin deficiency Exclusion of the probands left 3 individuals of each sex. When all sibships, including probands, were pooled, the ratio between individ

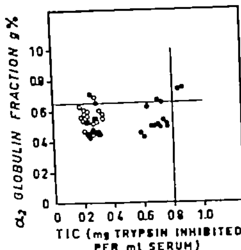


Fig. 23. Electrophoretic α_2-globulin values plotted against TIC in individuals with α_1 ntitrypsin deficiency (unfilled circles) and in children nd parents f affected individuals (filled circles)

TABLE II. *TIC (Mean ± S.D) and observed range in the subgroups studied Figures within brackets refer to number of individuals*

	Subgroups		TIC Mean ± S.D	TIC Observed range
TIC < 0.4	A proband	(14)	0.24 ± 0.03	0.18—0.34
	B parents	(1)	0.29	—
	C children	(0)	—	—
	D siblings	(6)	0.24 ± 0.03	0.15—0.31
	E ther relatives	(1)	0.25	—
	Σ A+B+C+D+E	(22)	0.25 ± 0.03	0.15—0.34
TIC 0.40—0.80	B parents	(5)	0.67 ± 0.06	0.56—0.72
	C children	(13)	0.68 ± 0.06	0.59—0.77
	D siblings	(27)	0.66 ± 0.08	0.49—0.77
	E ther relatives	(17)	0.60 ± 0.05	0.50—0.79
	Σ B+C	(18)	0.67 ± 0.06	0.56—0.77
	Σ B+C+D+E	(62)	0.67 ± 0.08	0.49—0.79
TIC 0.80—1.40	B parents	(0)	—	—
	C children	(0)	—	—
	D siblings	(8)	1.04 ± 0.08	0.92—1.16
	E other relatives	(14)	1.14 ± 0.09	1.00—1.35
	Σ B+C+D+E	(22)	1.10 ± 0.10	0.92—1.35
	CONTROLS	(100)	1.07 ± 0.12	0.84—1.40

2 individuals with increased α_2-globulins excluded.
2

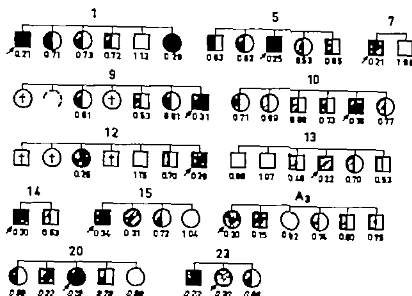


Fig. 24. Individual TIC in 12 sibships.

uals with and without α_1 antitrypsin deficiency was 18/35 against 6/35 when the probands were excluded

Table II summarizes the TIC values found in the different subgroups studied.

Fig 25 shows a pedigree of a family with 4 matings between a normal individual and one with intermediate TIC. TIC was determined in 13 children of these matings Of these the TIC was normal in 7 and intermediate in 6 All had normal α_1-globulin values.

In 2 families (7 and 12) TIC was determined in three generations. Complete pedigrees of these families are given in chapter VII The defect was traced in all three generations.

Fig 26 shows the immunoelectrophoretic pattern obtained with a specific α_1 antitrypsin antiserum in one individual with α_1 antitrypsin deficiency one with intermediate and one with normal TIC, each representing one of three families with α_1 antitrypsin deficiency The pattern of a pooled normal serum is included for comparison. The reduced electrophoretic mobility of α_1 antitrypsin in the 3 individuals with the deficiency state is obvious The electrophoretic mobility of the α_1 antitrypsin in the individuals with intermediate and normal TIC was the same as that in the pooled normal serum.

Paper electrophoresis of sera from individuals with intermediate TIC invariably revealed an α_1 band but never in sera from those with α_1 antitrypsin deficiency (fig 27) Sometimes the band appeared less intense than in normal serum but as a rule it was impossible to assign an individual to the intermediate group without first determining the TIC.

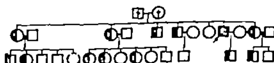


Fig. 25. Pedigree illustrating family 10

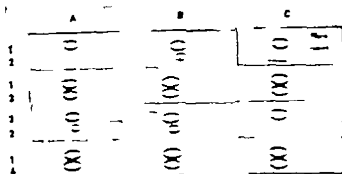


Fig. 26. Immunoelectrophoretic pattern in sera from members of three different families (A, B, C)

1 Normal pooled serum.
2 Serum with α_1-antitrypsin deficiency
3. Serum with intermediate TIC
4 Serum from normal family members.

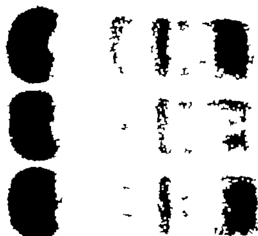


Fig. 27 Paper electrophoretic pattern in α_1-antitrypsin deficiency (above) serum with intermediate TIC (middle) and normal serum (below)

DISCUSSION

The results obtained corroborate the assumption based on earlier observations (Eriksson 1964) that three different levels of α_1 antitrypsin occur in α_1 antitrypsin deficiency families. In chapter III it was pointed out that α_1-antitrypsin activity is not influenced by the level of α_1-antitrypsin activity and therefore the reduction of TIC in the deficiency state is a reflection of the reduced level of α_1 antitrypsin activity. It is obvious that although TIC represents the sum of all trypsin inhibitors in serum it permits a rough identification of three different types of individuals in families of persons with α_1 antitrypsin deficiency. It is reasonable to assume that determination of the TIC is just as good a criteria for classifying an individual as the more elaborate and time consuming selective determination of α_1 antitrypsin described in chapter III.

From the work of Jacobsson (1955) it is known that an increase in α_1 antitrypsin activity often occurs in conditions accompanied by tissue destruction. From his work it is also known that a decrease of the activity due to urinary loss of the protein can occur in the nephrotic syndrome. The same holds true for advanced liver cirrhosis (unpublished observations). Both these conditions give rise to grossly abnormal paper electrophoretic patterns and typical clinical pictures. There is no reason to believe that either renal or liver disease is present in any of the family members investigated. From fig. 23 it is evident that in α_1 antitrypsin deficiency the level of α_1 antitrypsin is practically uninfluenced by an active process revealed by an increased α_1-globulin value. As a normal electrophoretic pattern was found among all relatives except 2 children and 2 parents external factors influencing the α_1 antitrypsin activity have been eliminated as far as possible. Therefore it is justified to conclude that the higher frequency of individuals with low or intermediate TIC in the families of affected individuals than among the controls (fig. 19) is due to genetic factors and not to external influences.

The sex distribution (20 males and 18 females) among the 38 individuals with α_1 antitrypsin deficiency studied was equal. This suggests that autosomal genes are responsible for the inheritance. Furthermore a completely X linked locus can be excluded as in families 1, 2, 7 and 10 (fig. 22) the atypical gene is transmitted from father to son which is never the case with a X linked gene. It can therefore be concluded that α_1 antitrypsin deficiency is not sex linked.

All data obtained in this material corroborate the hypothesis (Eriksson 1964) that α_1 antitrypsin deficiency is transmitted by a recessive gene. Let a design an atypical autosomal gene and A its normal allele. Then α_1 antitrypsin deficiency is a homozygous (aa) manifestation, the intermediate phenotype with TIC 0.4—0.8 mg/ml occurs in heterozygotes (aA) and the normal state in homozygotes (AA) for the nor

mal allele Thus α_1-antitrypsin defi ciency can be said to be recessively inherited with about the same justi fication as is the case with galactosemia and acatalasemia Six types of matings will then be possible, and of them all except mating type *aa*×*aa* were noted in the present material. Examples of mating type *AA*×*Aa* are found in the pedigree in fig. 25. Theoretically off pring of such a mating should be equally often *AA* as *Aa*. This was obviously the case in practice, for 7 of the 13 offspring had normal TIC and 6 intermediate TIC. Family 23 in fig 21 (complete pedigree in chapter VII) contains an example of mating type *aa*×*Aa* Offspring of such a mating should be *aa* and *Aa* in the ratio 1 1 There were only 8 offspring and none of them had normal TIC. With the exception of the mother of the proband in thi family all parents of indi iduals with α_1-antitrypsin def ciency had intermediate TIC (fig. 21) This is in line with what may be expected of a recessi e gene namely that parents of affected indi iduals should be heterozygotes (*Aa*) Of the offspring in mating type *AA* × *aa* illustrated in fig. 20 all were *aA* except two. These individ als had increased α_2-globulin values. This finding is more fully discussed later

In the 12 segregating sibships illu trated in fig. 24 there were 18 affected individual compared with 35 unaf fected. When applying Weinberg s proband method to these data there remained 6 affected and 35 unaffected. Ideally $\frac{1}{4}$ or 10.2 sibs hould be af fected. This deviation from the ex pected ratio can be ascribed entirely to chance (χ^2=2.30 d.f =1 0.20 > p > 0 10)

The TIC values chosen for limiting the intermediate phenotype were 0 4 and 0 8 mg/ml (fig 10) When defin ing the upper limit of TIC in the inter mediate phenotype more exactly the distribution of TIC among the normal controls, compared with that among known heterozygotes must be taken into account. Assuming a Gaussian distribution of TIC among the normals, 2.5 per cent of a normal population should have a TIC below 1 07 minus 2 S.D (0.83) and 0 15 per cent below 1.07 minus 3 S D (0 71) The observed range (0.85—1 40) did not quite agree with that calculated and thereby indi cated a non-gaussian distribution. Assuming the recessi e type of inherit ance suggested here, the known heterozygotes who must be used in defining TIC in the intermediate phenotype consist of parents and children of affected individuals. As in the controls, the observed and calculated ranges did not agree completely (table II) The actual range was 0 77—0.56 The TIC val e distinguishing the normal from the intermediate phenotype was there fore chosen intermediate the lowest value in the normal controls and the highest in the known heterozygotes In the calculation of the TIC range in known heterozygotes, individuals with increased α_2-values were excluded. The reason for this is obvious from fig. 23, which shows a strong correla tion between TIC and α_2-globulin value in these indi iduals. This means that

the phenotypic expression of the heterozygous genotype *Aa* is influenced by the presence of an "active process" revealed by the elevated α_2-globulin value.

It is probable that as in normals in carriers of one gene for α_1 antitrypsin deficiency (*Aa*) TIC can be raised by such stimuli as infections Whether this response is quantitatively comparable to that in normals is not known In contrast in individuals homozygous (*aa*) for α_1 antitrypsin deficiency TIC seemed to be almost unaffected by conditions with associated elevation of the α_2-globulin level

The highest TIC in individuals with α_1 antitrypsin deficiency was 0 34 mg/ml and the lowest intermediate TIC was 0 49 mg/ml (fig 19) The TIC value distinguishing homozygotes (*aa*) from heterozygotes (*Aa*) was taken as 0 40 mg trypsin inhibited per ml.

It is evident that determination of the TIC enables identification of the heterozygous carrier state in α_1 antitrypsin deficiency If individuals with increased α_2-value are excluded there will be no overlapping between normals and heterozygotes However in equivocal cases a family study is helpful in the classification of an individual TIC value.

The mean TIC in individuals classified as "known heterozygotes" was $0\ 67 \pm 0\ 06$ (S D) mg/ml. Table II shows the similarity of means and standard deviations in individuals with intermediate TIC among siblings ($0\ 66 \pm 0\ 08$ (S D)) and among other relatives ($0\ 69 \pm 0\ 05$ (S.D)) These individuals may be called "assumed heterozygotes" When all heterozygotes were taken together the mean TIC was $0\ 67 \pm 0\ 08$ (S D) against 1 07 mg/ml in the normal controls and 0 25 mg/ml in α_1 antitrypsin deficiency The mean TIC value in heterozygotes was thus nearly intermediate between that in normal individuals and that in individuals with α_1 antitrypsin deficiency $\left(\frac{1\ 07+0\ 25}{2}=0\ 66\right)$ This finding further supports the theory of intermediate expression in heterozygotes for α_1 antitrypsin deficiency

The electrophoretic migration rate of serum α_1 antitrypsin in atypical homozygotes (*aa*) is slightly lower than in normals In heterozygotes (*Aa*) the migration rate appeared to be normal (fig 26) One would expect the α_1 antitrypsin in heterozygotes to be a mixture of equal parts of an "atypical" α_1 antitrypsin caused by the action of gene *a* and a "typical" α_1 antitrypsin caused by the allelic gene *A* Though it is not known whether this really is so a refined electrophoretic technique (crossed antigen antibody electrophoresis) has revealed the presence of this "atypical α_1 antitrypsin" in both heterozygotes and normals (Laurell 1965) The possibility that the retarded electrophoretic mobility of α_1 antitrypsin in homozygotes for α_1 antitrypsin deficiency is caused by an aggregation product between "typical" α_1 antitrypsin and a substance x has been suggested (Laurell 1965) Ordinary immunoelectrophoresis is not sensitive enough to reveal this aggregation in heterozygotes and normals.

In chapter I it was pointed out that

the original normal material studied consisted of 103 individuals (51 men and 52 women). In 3 of them the TIC fell distinctly outside the range of the other values (fig. 4 and fig. 19). On the other hand they fell within the heterozygous range accepted here and were therefore regarded as representing the heterozygous state. Family investigations in one of these cases lent support to the assumption of heterozygosity. The question of the frequency of heterozygotes for the allele causing α_1-antitrypsin deficiency is dealt with in chapter VI. The frequency found in the present material, comprising 103 individuals, was $^1/_{34}$.

SUMMARY

Assay of total trypsin inhibitor capacity (TIC) was used in a study of 14 families with a case of α_1 antitrypsin deficiency in each. Including controls, all together 213 individuals were studied.

The distribution of TIC among family members was found to be trimodal. In the group with the lowest level, thought to represent the homozygous state of α_1-antitrypsin deficiency, the mean TIC was 0.24 mg trypsin inhibited per ml serum. In the intermediate group, thought to represent the heterozygous state, the corresponding value was 0.67. In normal family members the mean of 1.10 compared with 1.07 mg/ml in 100 normals, was found.

The range of TIC in heterozygotes was defined as 0.40—0.80 mg trypsin inhibited per ml serum. There was no overlapping of the three groups of TIC values after exclusion of individuals with increased α_1-globulin fraction.

TIC in heterozygotes may increase in response to suitable stimuli but not in homozygotes.

α-antitrypsin deficiency seems to be a genetic defect transmitted by a recessive autosomal gene.

Chapter VI

THE FREQUENCY OF THE GENE FOR α_1 ANTITRYPSIN DEFICIENCY

INTRODUCTION

In the preceding chapter (V) the α_1 antitrypsin deficiency was shown to be transmitted by a recessive gene. In a sample of 103 presumably normal individuals 8 representing the heterozygous state were detected. To get a more accurate idea of the frequency of the gene in a Swedish population the number of homozygotes for α_1 antitrypsin deficiency was estimated in a larger sample consisting of 6 995 individuals.

MATERIAL AND METHODS

In 1962 the Swedish Board of Health arranged mass screening of a population of 100,000 people for asymptomatic disease. The screening project, in which a chemical test battery was used, was started in Värmland in October 1962. Värmland is a county in the western part of Sweden with nearly 300 000 inhabitants. All inhabitants 25 years of age or more were invited to take part in the *project*. During 1964 inhabitants of three districts (*fig. 28*) (*Eks härad, Gustav Adolf, Råmmen and Norra Råda*) were examined. Of the inhabitants invited 82 per cent co-operated. Of these blood samples were taken from 6,995 (70 per cent of the population). These sera were studied by paper electrophoresis and by determination of trypsin inhibitor capacity.

Paper electrophoresis was performed according to Laurell et al. (1956). Only one strip was run and the various fractions were therefore not eluted.

Total trypsin inhibitor capacity (TIC) was determined as described in chapter V.

RESULTS AND DISCUSSION

In 25 of the 6 995 paper electrophoretic strips examined repeatedly with the naked eye the pattern suggested α_1 antitrypsin deficiency. These sera were selected for further study. Determination of TIC confirmed the suspected homozygosity for α_1 antitrypsin deficiency in *4 (2 males and 2 females) of* these 25 individuals. Of the remaining 21 individuals, 15 were found to represent the *heterozygous* state of α_1 antitrypsin deficiency and 6 were normals, in whom α_1 antitrypsin deficiency had been suspected owing to inferior technical quality of the paper electrophoretic strip.

The frequency *of homozygotes for* the gene causing α_1 antitrypsin deficiency in the population of 6 995 individuals was thus $\frac{4}{6995} = \frac{1}{1749} = 0.00057$

The frequency (*q*) of the recessive allele (*a*) causing α_1 antitrypsin deficiency can be calculated from the

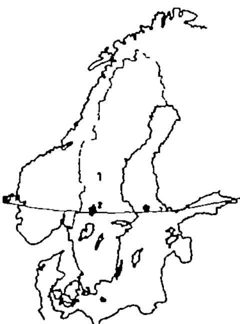


Fig. 25 The black area (2) indicates the district covered by the population survey. 1 Sweden 2 Malmö

known frequency of homozygotes (q^2). Since $q^2 = \frac{1}{1749}$, $q = \frac{1}{42} = 0.024$ and the frequency of the normal allele (A) $p = \frac{41}{42} = 0.976$. On the assumption of random mating in the district studied, application of Hardy–Weinberg's law gave the frequency of heterozygotes $(Aa) = 2\ p\ q = 2 \times \frac{1}{42} \times \frac{41}{42} = \frac{1}{21} = 0.047$

This figure is based on a large sample probably representative of the entire population of Sweden. On the other hand, the number of homozygotes was so small that it might be questioned whether it represents the true frequency of the gene. As shown in chapter VII, there is an *overmorbidity* of chronic obstructive pulmonary disease in individuals with α_1-antitrypsin deficiency. Since the life expectancy of these individuals is presumably decreased, the homozygote frequency given here might be fictitiously low. On the other hand, the heterozygote frequency is in reasonable agreement with the figure of $^1/_{33}$, calculated in the preceding chapter (V). This figure is based on a small material consisting of only 103 individuals. The gene frequency should preferably be determined by selection of the intermediate *phenotype in the present material.* For technical reasons this was not possible since demonstration of heterozygosity requires determination of TIC. Heterozygosity cannot be demonstrated by mere inspection of paper electrophoretic strips (chapter V).

The frequency (0.024) of the gene for α_1-antitrypsin deficiency is of such an order of magnitude that a restricted distribution of the gene seems improbable. As a matter of fact cases of α_1-antitrypsin deficiency have been found in all parts of Sweden. Cases have also been reported from Switzerland (Lopez et al., 1965) and from U.S.A. (Kueppers et al., 1965). One of the cases presented in chapter VII (4) was born in Estonia.

SUMMARY

Four cases of α_1 antitrypsin deficiency were detected by inspection of paper electrophoretic strips and subsequent determination of total trypsin inhibitor capacity of sera from 6 995 persons. The calculated frequency of the gene causing α_1 antitrypsin deficiency was 0 024 $\left(\frac{1}{42}\right)$ and the calculated heterozygote frequency was 0 047 $\left(\frac{1}{21}\right)$

Chapter VII

α_1 ANTITRYPSIN DEFICIENCY AND CHRONIC OBSTRUCTIVE BRONCHO-PULMONARY DISEASE

INTRODUCTION

Examination of five patients with α_1 antitrypsin deficiency revealed chronic obstructive pulmonary disease in three of them (Laurell and Eriksson, 1963) Family studies in one of these cases disclosed that three siblings had obstructive pulmonary disease with early onset (Eriksson, 1964) Two siblings also had α antitrypsin deficiency The third sibling had died.

This chapter is concerned with a survey of the clinical findings in 37 cases of α_1-antitrypsin deficiency with special reference to lung function. It also includes data on the fibrinolytic activity in the serum and the concentration of the electrolytes in the sweat in some of the cases.

MATERIAL

1 *The hospital material* consisted of 33 patients. [illegible] homozygotes for α_1-antitrypsin deficiency Twenty four of the 33 patients were probands, the remaining 9 had been discovered in family studies. Of the 24 probands, 13 had been found at examination of routine electrophoretic strips in 1962—1964 t the Central laboratory Malmö General Hospital The remaining cases were found t the Central laboratory Lund (3 cases) Central laboratory V2 J5 (4 cases) Department of medicine, Kristianstad (1 case) Central laboratory Boden (1 case) and Osterbotten Sanatorium (1 case) T cases were also traced on examinatio of the files of the Central laboratory Lund. Of these 33 cases, the following ha been described in previous publications: Nos. 11 13, 14 21 nd 20 (Laurell nd Eriksson, 1963) and family 12, including cases 13, 14 and 15 (Eriksson, 1964)

All of the patients except Nos. 5 and 20 were examined personally by the uthor t the department of medicine, Malmö General Hospital. In cases 5 and 20 the hospital records contained sufficient data to allow firm clinical diagnosis.

2 *Additional case* (A_1—A_4) These four cases were discovered during the population survey in Värmland (chapter VI) These cases will be studied further In 2 f these 4 cases the data were sufficient to make firm clinical diagnosis.

3. *Fibrocystic disease f the pancreas.* Sera from three children were examined for TIC.

METHODS

The *history* was taken with special reference to earlier respiratory infections, cough, sputum nd dyspnea. Special ttention wa given to the chronology of the symptoms. To t

tempts were made to estimate the amount of sputum coughed up. A detailed inquiry was made into the family history for any diseases of the lungs or allergy in the family

Grading of dyspnea The patients were classified according to exercise tolerance The four group system suggested by Mitchell and Filley (1964) was used

The general clinical investigati n included a routine physical examination, electrocardiography (leads I II III aVR aVL, aVF and V_1—V_6) determination of hemoglobin (Hb) red blood cells (RBC) white blood cells (WBC) and a differential count. Urine was xamined for protein (Albustix) and glucose (Clinistix) Counts were also made of the total number of eosinophils. Normal value 75—400 per mm³ blood.

Special laboratory investigations

Bromsulphalein retention was d termined according to Gaebler (1945) 5 mg/kg body weight bromsulphalein was given intravenously and the r tention determined after 30 minutes. Normal value 2—10 per cent retained.

Sodium in sweat was determined according to Gibson and Cooke (1959) Normal value for adults 25—75 mEq/l.

Assays of fibrinolytic act ity Th fibrinolytic activity was determined on unheated fibrin plates in th manner described by Nilsson and Olow (1962) The activity is expressed as the diameter product (mm²) of the lysed zones. N rmal values for citrated plasma 0—50 mm² lysed area and for eugl bulin precipitate 0—70 mm lysed are

Antiplasmin wa d termined according to Nilsson et al. (1961) Normal value 60—140 per cent.

Lung function te t The t rminology used is mainly that suggested by Pappenheime (1950) The bbreviations used are listed below

VC (l)	Vital capacity
$FEV_{1.0}$ (l)	Forced expirat ry v lume in 1 sec
FEV %	$\frac{FEV_{1.0}}{VC} \times 100$
FRC (l)	F nctional residual capacity
TLC (l)	Total lung capacity
LCI	Lung clearance index (Becklake 1952)
PaO_2 (mm Hg)	Arterial oxygen tension
$PaCO_2$ (mm Hg)	Arterial carbon dioxide tension.

All volumes are given at ambient temperature and pressure saturated (ATPS) Approximate BTPS values may be obtained by multiplying the volumes given here by a factor of 1 10 $FEV_{1.0}$ was determined with a B rnstein spirometer as modified by Berglun d et al. (1963) The normal values used for $FEV_{1.0}$, FEV and VC are those given by these authors. FRC was measu ed with the open circuit technique of Darling et al. (1940) as modified by Bouhuys et al. (1956) The normal values are taken from Grimby and Söderholm (1963) The distribution of inhaled gas in the lungs was judged from the lung clearance index according to Becklak (1952) i.e from the volume of oxygen required to wash o t N_2 (down to 2) from 1 l of FRC. Th normal values for LCI have been obtained from Bouhuys (1963) PaO_2 was measured with a modified Clark electrode (1956) Normal alue 85—104 mm Hg $PaCO_2$ was measured according to Severinghaus and Bradley (1958) Normal value 39—43 mm Hg.

In order to judge to what ext nt the obstructive pulmonary disease found i the patients could be influenced by bronchodilato ry drugs, aminophylline w s gi n intrave n usly in a dose f 250 mg and the spirometry repeated aft r 15 minutes.

In mo t patie ts the lung function te ts were perf rmed at the Department f Clinical Physiology Malmö Th results are g en in table IV When lung function tests w re performed at other ho pit ls, th results re given in the case report. Care wa taken to investigat patients when the were in optimal cond tion nd had no acut complicatio of th ir disease When patie t wa examined repeatedly o ly the ex minatio that ga e the best result wa con idered

Radiological x mination

Chest adiography including po tero-anterior and l teral projectio had been done in all

cases. Chest X rays had been taken in deep inspiratio

Pulmonary angiography was perf rmed with tip occluded Cournand catheter introduced through cubital ein and placed in the pulmonary artery

Definitions

Emphysema, hronic bronchitis and bronchial asthma are defined according to the proposals of the World Health Organization Committee on chronic cor pulmonale (1961)

Emphysema is defined in anat mical terms: Emphysema is condition of the lung har acterized by increase beyond the normal in the size of air spaces distal to the terminal bronchiole with destruction of their walls

Chronic br nchitis is defined in *clinical terms*: Chronic bronchitis is chronic or recurrent increase bov the normal in the volume of bronchial mucous secr tion, sufficient to cause expectoratio when thi is not due to localized broncho-pulmonary disease. The words chronic or recurrent ma be furthe defined present on most day during l lea t three mo ths in each of tw successive ears

Asthma bronchial Asthma refers to the condition of subjects with wide-spread na row ng f the bronchial irw ys, which changes its severity er short periods of time either spontaneously or under treatment, nd is not due to cardiovascular disease. The term *asthmatic bronchitis* is used here to describe patients fulfilling the criteria for chronic bronchit with predominant broncho-spa tic reversible component

Primary emphysema denotes the condition in patients ho develop persistent irway obstruction without ny history of chronic bronch tis nd who therwise fulfil the criteria given abov for emphysema

The term *cor pulmonal (hronic)* is used to describe congestiv heart failure obviously secondary to chronic obstructive pulmonary disease It does not include the involvement of the left entricle before the development of failure (Stuart-Harris and Hanley 1957) (table III.)

Right ventricular hypertrophy (RVH) nd right atrial hypertrophy (RAH) wa judged according to the electrocardiographic criteria given by Goldman (1953) (table III.)

Blood eosinophilia was said to be present (+) when the total eosinophil count exceeded 600/mm² or when the eosinophils in the dif ferential count repeatedly were found to represent more than 7 per cent. Absence of eosinophilia was marked with 0 (table III)

Radiological signs of emphysema

The diagnosis f emphysema wa based on the clinical history the physical examination, the lung function tests and the radiological findings That the presence of morphological emphysema is characterized by visible changes in the pulmonary vasculature has been well documented (for review see Bates and Christie, 1964) I the evaluation of the vascular tree the criteria given by Laws nd Heard (1962) were used These uthors found that reduction in the calibre and number of the peripheral pulmonary arteries, often with a increased transradiancy of the background due to reductio in the vascular bed, were the most reliable radiological signs of emphy sema These igns were estimated from postero-anterior nd lateral iews. I some cases selective pulmonary angiography was performed. The films were examined independently by tw radiologists. One of them classified the degree of emphysema absent (0) moderate (+) or severe (++) (See table III) I addition notes wer made of any wide-spread "fibrosis chronic pneumonitis or other chronic parenchymal inflamm tory changes (Burrows et al 1964) All patients were then subgrouped ccording to Scarrow (1964)

Group I The chest radiograph shows little evident variation from the normal.

Group II The radiological finding i one of widespread diffuse emphysema.

Group III There is mixture of chronic inflammatory changes in the lung fields interspersed with area of radiologically normal or hyperaemic lung and areas of emphysema.

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Group IV Those cases in which there are extensive chronic inflammatory changes in the lung fields, and these are the dominant features of the radiograph.

The bronchographic findings were interpreted according to Simon (1958)

Presentation of the material

All probands are presented chronologically according to date of birth with males first. The figures above the pedigrees refer to running family number and cases belonging to the family. In cases where no pedigree is available the family number is given within brackets. Every patient is presented with a short case report giving only information relevant to the diagnosis. Results of clinical and laboratory investigations, lung function tests and roentgen examinations are given mainly in tabular form (tables III IV V). A family history is given only if it is relevant to the proband's disease. A probable diagnosis based on anamnestic, clinical, radiological and laboratory data is given at the end of each case report

Key to pedigrees


Male normal TIC


Intermediate TIC

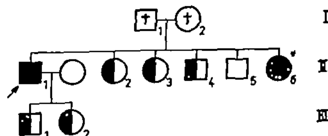

α_1-antitrypsin deficiency proband


pulmonary disease


Female normal TIC


TIC not determined

CASE REPORTS



Family 1

Case 1 (II_1) A mechanic, born in 1901 His maternal grandfather had had chronic bronchitis and a sister has bronchial asthma and emphysema

(case 2) During an attack of influenza in 1918 he had bilateral pneumonia, and in 1920 he had a recurrence of the pneumonia Since 1935 he had had protracted attacks of often purulent bronchitis, during which he brought up large amounts of expectorate. Since 1950 he had had at least 6 attacks of pneumonia and increasing dyspnea Since 1945 he had had moderate hyper tension without signs of enlargement of the heart or renal injury *Lung func tion tests* (table IV) showed obstructive impairment of pulmonary function without signs of reversibility *Pulmonary angiography* (fig. 29) showed a substantial loss of parenchyma and only a parse vascular bed in the lower lobes. The relatively straight course of the essels in the right lower lobe sug gests the occurrence of emphysema bullae there. *The total eosinophil count* was 700/mm

Diagnosi Chronic bronchitis and em physema.

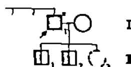


Fig. 29 Pulmonary angiogram in case 1. (1961)

Case 2 (II₂) A female, born in 1915 Si ter of the patient in case 1 As a child he had had eczema but no def inite allergic manifestation until 1945 when typical bronchial sthma made it ppearance In 1950 and 1958 she was treated f r pneumonia. During the last few years she had had more or less chronic asthma with several attacks per month. Several precipitating agents are known, but hyposensitization has failed to produce the desired effect. Since 1957 she has had a progressive effort dyspnea. She has lost about 10 kg body weight since 1900 She has been treated with small doses of ster oids during the last few years. She has no phlegm and is not liable to respira tory tract infections. *Lung function tests* (table IV) showed obstructive im pairment of function with a certain reversibility *Chest X ray* showed a marked deficiency of the vasculature to the lower lobes. The total eosinophil count was 1800/mm³

Diagnosis Bronchial asthma and em physema

Family 2



Case 3

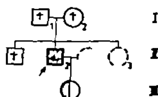
Case 3 (II) A gardener born in 1903 In 1952 he had pneumonia and has since had protracted recurrent periods of bronchitis, especially during the



Fig 30 Bronchogram (lingular segment 1 ft lung) in case 3 (1963)

winter months. He has been dyspnoic since 1952 but during the last few years he had become much more breathless than before In 1963 he was admitted to hospital because of pneumonia of the middle lobe *Lung function tests* (table IV) showed obstructive ventilatory impairment He was not examined for reversibility Right sided *bronchography* showed bronchitic changes (fig 30) and *chest X ray* reduced vasculuture to the lower lobes, a finding compatible with pulmonary emphysema He had no blood eosinophilia

Diagnosis Chronic bronchitis and emphysema.



Family 3 Case 4

Case 4 (II_2) A veterinary surgeon born in 1904 in Estonia A brother (II_1) died at 40 years of age from advanced emphysema (hospital records not available) The patient's symptoms made their first appearance around 1950 with effort dyspnea as a dominating symptom During recent years he has had 2 or 3 episods of bronchitis per year with marked deterioration of his condition Examination revealed clubbing of the fingers and marked barrel chest *Lung function tests* (table IV) showed severe obstructive impairment without signs of reversibility *Chest X ray* demonstrated sparsity of vessels in the peripheral lung fields, especially in the lower lobes There was no blood eosinophilia

Diagnosis Primary emphysema complicated by chronic bronchitis

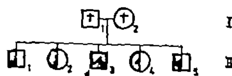
Case 5 A porter born in 1905 In 1940 he was operated upon because of appendicitis, and the postoperative course was complicated by pneumonia. In 1948 he had incipient symptoms with cough and dyspnea In 1950 he was ad mitted to hospital because of chronic bronchitis. His VC that year was 3.5 l and R.B.C. 4.8 mill/mm³ His disease steadily progressed during the follow ing years with recurrent infections, purulent expectorate and increasing dyspnea *Chest X ray* in 1960 (fig. 31) showed broad, fibrotic hila and signs of emphysema and bronchiectasis. Clubbing of the fingers was noted in 1960 In 1963 his VC had decreased to 2.5 l nd FEV was 0.8 l. Polycythae mia with R.B.C. 6.5 mill/mm³ aper vened. Finally cor pulmonale devel oped. The oxygen tension was low but the CO_2-tension was normal. In September 1963 he was placed in a respi rator He died soon afterwards from pulmonary embolism. Post mortem examination revealed a bullous em physema and cylindric bronchiectasis in the lower lobes.



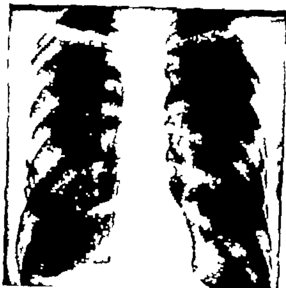
Fig. 31 Chest X-ray in case 5. (1960)

Diagnosis Chronic bronchitis with bronchiectasis and bullous emphy sema.

Family 5



Case 6 (II 3) A clerk, born in 190 The m ther had died of pneumonia. Since 1948 he ha had increasing dyspnea or exertion which gradually progressed to dyspnea group IV He has n ver had pneumonia nd seldom bri gs up ny phlegm. In 1031 he was operated upon for perforating ulcer and in 1032 he was submitted to gastric resection ad modum B llroth II. Since then he has lost about 20 kg body weight but he has not had any anaemia. He is a smoker with a daily consumption of about 20 cigarettes. *Lung function tests* (table IV) howed severe obstructive ventilatory impair



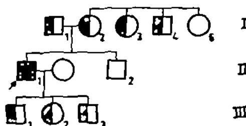
ment without signs of reversibility *Chest X ray* (fig 32) showed marked deficiency of the vasculature to the lower lobes and middle lobe. The total eosinophil count was normal.
Diagnosis Primary emphysema.

Fig 32 Chest X ray in case 6 (1963)

(Family 6)

Case 7

Case 7 A manager born in 1908 The father may have had asthma and a brother has symptoms of allergic rhinitis In 1942 symptoms appeared with a dry cough in the beginning and, since 1944 also with progressing breathlessness In 1950 asthmatic symptoms supervened They were precipitated particularly by infections with wheezing and attacks of increasing dyspnea Since 1958 his effort dyspnea had increased markedly so that he was unable to do anything except office work. Since 1959 he had been treated with small doses of steroids. At *lung function examination* in 1958 when he was relatively free from symptoms, the VC was 3 l (60 % pred) $FEV_{1.0}$ 1 0 l (27 % pred) TLC 8.8 l (160 % pred) and FRC 6 8 l (153 % pred) When admitted to this hospital in 1964 he was troubled by severe asthmatic symptoms so that no lung function tests could be performed. PaO_2 was 50 and $PaCO_2$ 41 mm Hg He had lost 10 kg in weight since 1958 *Chest X ray* showed in addition to hyperinflation a deficiency of the vasculature to the lower lobes He had had a slight blood eosinophilia
Diagnosis Asthmatic bronchitis and emphysema.



Family 7

Case 8

Case 8 (II_1) A life insurance agent born in 1916 He had always felt well until 1945 when he began to be dyspnoic on exertion and on exposure to

dust. He had no special tendency to respiratory tract infections. He had previously worked as a farmer but as his dyspnea progressively became worse he had to give up that occupation in 1954 and he is now working as an insurance agent. His main complaint has always been dyspnea on effort, and he cannot walk more than 100 meters on even ground without becoming dyspnoic. *Lung function tests* (table IV) showed obstructive ventilatory impairment without signs of reversibility. *Chest X ray* showed a reduced peripheral vascular pattern. The total eosinophil count was normal.
Diagnosis Primary emphysema.

(Family 8) Case 9

Case 9 A joiner born in 1916. A sister had died from pneumonia at 20 years of age. The patient's symptoms appeared in 1934 after respiratory tract infection (pneumonia?) with successively increasing dyspnea on exertion, particularly in cold and dusty air. His resistance to infections does not appear to be decreased but he sometimes coughs up phlegm in the morning when wheezing may also occur. His main complaint is, however dyspnea on effort, which has disabled him more and more. He has lost 17 kg since the onset of the disease. *Lung function tests* (table IV) showed severe obstructive ventilatory insufficiency without signs of reversibility. *Pulmonary angiography* (fig. 33) showed a markedly retarded flow to the lower lobes and middle lobe as well as narrowing of the vessels. No bullae could be demonstrated at tomography. *Bronchography* showed nothing abnormal. There was no eosinophilia.
Diagnosis Primary emphysema

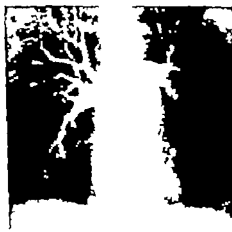
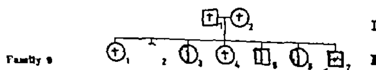


Fig. 33. Pulmonary angiogram in case 9. (1963)

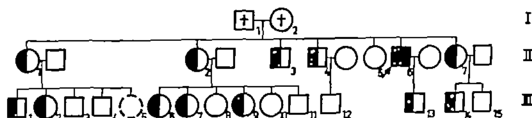


Family 9 Case 10

Case 10 (II_7) A foundryman, born in 1918. In 1952 he had duodenal ulcer. His present disease made its appearance in 1954 with an infection of the

upper airways with obstinate cough and dyspnea The following years he frequently had infections of the respiratory tract and rapidly increasing dyspnea. As early as 1956 chest X ray showed signs of marked emphysema His VC that year was 2 9 l In 1962 asthmatic symptoms supervened with allergy to dust, but no blood eosinophilia In 1962 he could no longer go out because of dyspnea. In 1964 cor pulmonale developed with massive oedema. He had moderate hypercapnia ($PaCO_2$ 56 mm Hg) He responded well to dehydrating treatment but he is now a respiratory invalid and confined to bed. Since 1956 he has lost 12 kg body weight

Diagnosis Probably a primary emphysema with complicating chronic bronchitis.



Family 10 **Case 11**



Fig 34 Bronchogram (left lung) in case 11 (1962)

Case 11 (II_6) A pastrycook, born in 1919 In 1945 he had a slight attack of pneumonia and in 1955 a recurrence. Since then he has had infections of the respiratory tract at fairly short intervals, especially during the winter months during which he often brings up large amounts of purulent matter but he has had no hemoptysis. As early as 1955 chest X ray had shown signs of bronchiectasis. Since 1961 after acute tracheobronchitis he has become worse with dyspnea and loss of body weight. *Bronchography* in 1962 (fig 34) showed generalized ectatic widening of the bronchi on both sides. *Lung function tests* (table IV) showed astonishingly good values with only slight obstruction and normal blood gas ten

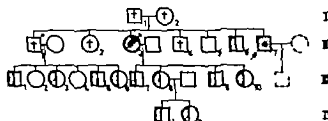
sions. *Chest X ray* showed a slight reduction of the vasculature to the lower lobes. There was no eosinophilia.
Diagnosis Chronic bronchitis with generalized bronchiectasis. The evidence for the presence of emphysema is equivocal.

(Family 11) Case 12

Case 12 A petrol pump attendant, born in 1924 Since about 1945 he had recurrent respiratory tract infections, especially in the spring and autumn with a productive cough. Since 1954 he had progressive dyspnea on exertion which became worse during infections. During the periods of infection he often had bronchospastic symptoms with wheezing. Because of his pronounced breathlessness he was pensioned off in 1962 Since then he has been treated with steroids, but without any effect. His *lung function values* (see table below) have been fairly constant throughout the years without any appreciable fluctuations suggesting severe, mainly irreversible obstructive ventilatory insufficiency

year	VC	$FEV_{1.0}$	FEV %
1961	3.3	0.9	27
1962	3.0	0.8	27
1963	3.1	0.8	25

Chest X ray showed reduced vasculature to the lower lobes. There was no blood eosinophilia.
Diagnosis Asthmatic bronchitis and emphysema.



Family 13

Case 13 (II_7) A painter born in 1925 In 1944 he had a pneumonia. Chest X ray in 1954 and 1957 revealed nothing remarkable In 1959 he began to have dyspnea on exertion. This symptom steadily progressed and was accentuated at respiratory infections during the autumn and winter months. Otherwise he had no cough or phlegm and his dominating symptom was dyspnea on effort Since 1962 he has been unable to work. Since the onset of the disease he has lost about 10 kg body weight. The *lung function tests* showed severe obstructive impairment without signs of reversibility (table IV) *Chest X ray* showed marked deficiency of the vasculature to the lower lobes (fig. 3a) He had no blood eosinophilia.
Diagnosis Primary emphysema.



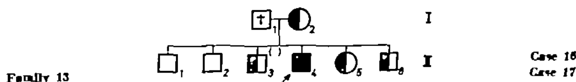
Fig. 35 Chest X ray in case 13. (1963)

Case 14 (II_3) A woman, born in 1915 She is the sister of the patient in case 13 She had obstinate bronchitis in 1932 Since 1953 she had noticed increased dyspnea on exertion with wheezing especially in cold weather From 1956 on she had obstinate infections with fever productive cough and increasing dyspnea during the winter months. Several agents are known to give her asthmatic attacks. She has *been treated by steroids periodically* since 1960 *Lung function tests* (table IV) showed obstructive ventilatory impairment without signs of reversibility *Chest X ray* showed a deficiency of peripheral vasculature. There was no blood eosinophilia.

Diagnosis Asthmatic bronchitis and emphysema.

Case 15 (II_1) A painter born in 1909 He was the brother of the patients in cases 13 and 14. Apart from diphtheria during childhood he had felt well until 1945 when he noticed dyspnea on exertion. From 1947 his dyspnea progressed rapidly with acute exacerbations in association with infections. Infections were often accompanied by asthma but his main complaint was dyspnea on effort. He had no known history of allergy Various cutaneous tests for allergy were negative. He died in 1954 after several years of disabling dyspnea and frequent infections with symptoms of asthma. He was not examined post mortem and no blood sample has been available from this patient. *Chest X ray* showed signs of emphysema.

Diagnosis Probably a primary emphysema complicated by asthmatic bronchitis.



Case 16 (II_4) A lorry driver born in 1928 A brother of the patient (case 17 II_3) had had spontaneous pneumothorax He had felt well until 1958 when he fell ill with pneumonia Since that infection he has had protracted bronchitis, particularly during spring and autumn and rapidly progressing dyspnea In 1961 he had *left sided* spontaneous pneumothorax with collapse of the entire lung *Tomography* revealed bilaterally large bullae with



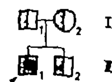
Fig. 36 Bullous emphysema in case 16 (right lung) 1 operation in 1964

out any demonstrable connection with the bronchial tree at *bronchography* Left sided thoracotomy was done in September 1961 which revealed pronounced bullous emphysema. The largest bullae were excised. Operation had a fairly good effect on the patient s subjecti e ymptoms. During 1963 the patient had 2 further attacks of right sided pneumothorax, and he was again operated upon in 1964 with right sided thoracotomy at which the largest bul lae in that lung were extirpated. Fig. 36 hows the extensi e bullous emphy sema seen at the operation in 1964 *Lung function tests* in September 1963 are given in table IV He has a severe obstructive ventilatory insufficiency

Diagnosis Primary bullous emphysema.

Case 17 (II_3) A butcher s assistant, born in 1927 brother of the patient in case 16. He had always been in good health until 1961 when he felt ill with left sided spontaneous pneumothorax. Thoracoscopy revealed a couple of em physema bullae on the anterior surface of the lower lobe. After drainage the lung expanded and the roentgenograms showed no signs of generalized emphysema. The patient has since felt well and is not troubled by cough or dyspnea. No lung function tests had been performed.

Diagnosis Local emphysema blebs without signs of generalized emphy sema

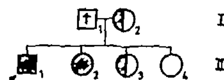


Family 14

Case 18

Case 18 (II_1) A carpenter born in 1942. Examination because of slight hypertension revealed that the patient was homozygous in respect of α_1 antitrypsin deficiency. He had no history of pulmonary disease and *roentgen examination* and *lung function tests* gave normal results.

Comment No signs of chronic obstructive broncho-pulmonary disease



Family 15

Case 19
Case 20

Case 19 (II_1) A metal worker born in 1939 Except for scarlatina as a child *he had always felt well. In 1950 he had* right sided spontaneous pneumothorax and 1961 he was treated for pneumonia of the right lower lobe. In January 1964 he again had right sided spontaneous pneumothorax *Chest X ray* revealed in addition to pneumothorax generalized fine nodular parenchymal changes of the type seen in pneumoconiosis or sarcoidosis Biopsy ad modum Daniels showed unspecific lymphadenitis. In April 1964 he had right sided pneumonia and in May 1964 a recurrence *of right sided pneumothorax* with total collapse of the lung Thoracotomy with decortication revealed a series of emphysematous bullae of varying size in the apical part of the right upper lobe and smaller bullae also in the middle lobe. The largest bullae were extirpated After operation the lung expanded and his general condition *has since been satisfactory His lung function* impairment (table IV) was considered to be of combined restrictive and obstructive type, but predominantly obstructive There were no signs of reversibility after aminophylline

Diagnosis Bullous disease of the lung probably a part of developing generalized emphysema.

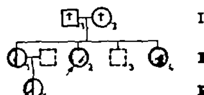
Case 20 (II_2) A female, born in 1941 She is the sister of the patient in case 19 She has always been in good health and physical examination and chest X ray revealed nothing abnormal. No lung function tests were performed.

Comment No evidence of chronic obstructive broncho-pulmonary disease.

(Family 16)

Case 21 A female, born in 1887 Rheumatoid arthritis appeared in 1933 and was afterwards her main trouble. Since 1956 she has been taking Butazolidin more or less continuously In 1957 she had acute cholecystitis. At that time spontaneous fractures of several thoracic vertebrae were detected. In 1961 and 1962 she had ulcers of the stomach. Laboratory studies in 1962 revealed the presence of a malabsorption syndrome with hypocalcemia and steatorrhea. She has been treated with D vitamins. In addition she has cardiosclerosis with slight cardiac decompensation. Repeated *chest X rays* have failed to reveal any signs of emphysema but have shown moderate pulmonary congestion. The *lung function tests* revealed slight combined restrictive and obstructive insufficiency and arterial oxygen tension was reduced to 72 mm Hg probably due to her cardiac decompensation.

Comment No clear evidence of obstructive pulmonary disease was noted in this patient.



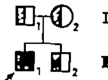
Family 17

Case 22 (II_1) A female, born in 1895 Her father had died at 63 years of age from pneumonia. A brother (II_3) had symptoms of bronchitis (he has not been examined) A sister (II_4) is the patient in case 23

The patient had had slowly progressive effort dyspnea since 1930 She had, however been able to work as a midwife until 1955 Since then her dyspnea has advanced and she is now in dyspnea *group IV Since 1960 she has* lost 5 kg body weight. In 1959 she had duodenal ulcer Her symptoms progress considerably during damp weather and during infections of the respiratory tract. She has never had pneumonia with certainty *Lung function tests* (table IV) showed obstructive ventilatory impairment without signs of reversibility *Chest X-ray* showed marked deficiency of the vasculature to the lower lobes and middle lobe. Total eosinophil count was normal. *Diagnosis* Primary emphysema.

Case 23 (II_4) A female, born in 1903 She is the sister of the patient in case 22. She had no history of dyspnea or phlegm but had had repeated upper respiratory tract infections with frequent infections of the sinuses. *Chest X ray and lung function tests* showed nothing abnormal.

Comment No evidence of chronic obstructive broncho-pulmonary disease.

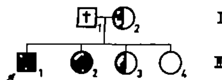


Family 14

Case 18

Case 18 (II_1) A carpenter born in 1942 Examination because of slight hyper tension revealed that the patient was homozygous in respect of α_1 antitrypsin deficiency He had no history of pulmonary disease and *roentgen examination* and *lung function tests* gave normal results.

Comment No signs of chronic obstructive broncho-pulmonary disease.



Family 15

Case 19
Case 20

Case 19 (II_1) A metal worker born in 1939 Except for scarlatina as a child he had always felt well In 1956 he had right sided spontaneous pneumothorax and 1961 he was treated for pneumonia of the right lower lobe. In January 1964 he again had right sided spontaneous pneumothorax. *Chest X ray* revealed in addition to pneumothorax generalized fine nodular parenchymal changes of the type seen in pneumoconiosis or sarcoidosis Biopsy ad modum Daniels showed unspecific lymphadenitis. In April 1964 he had right sided pneumonia and in May 1964 a recurrence of right sided pneumothorax with total collapse of the lung Thoracotomy with decortication revealed a series of emphysematous bullae of varying size in the apical part of the right upper lobe and smaller bullae also in the middle lobe. The largest bullae were extirpated. After operation the lung expanded and his general condition has since been satisfactory His *lung function* impairment (table IV) was considered to be of combined restrictive and obstructive type but predominantly obstructive There were no signs of reversibility after aminophylline

Diagnosis Bullous disease of the lung probably a part of developing generalized emphysema.

Case 20 (II_2) A female born in 1941 She is the sister of the patient in case 19 She has always been in good health and physical examination and chest X ray revealed nothing abnormal. No lung function tests were performed

Comment No evidence of chronic obstructive broncho pulmonary disease

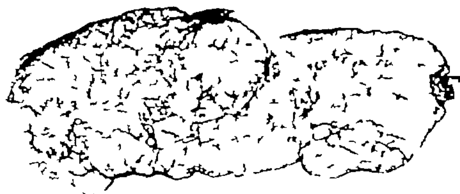


Fig. 27 Lung section from case 27 Prepared according to Gough and Wentworth (1960)

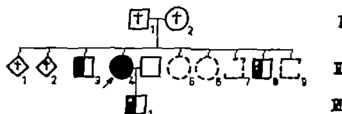
1952 In recent years she had had infections at shorter intervals, as a rule with purulent sputum. She had pneumonia several times every year from 1961 to 1964 She reported that she had always been dyspnoic but the dyspnea had became much worse since 1958. Since the onset of the disease in 1952 she had lost 20 kg. In 1960 she had bleeding gastric ulcer *Pulmonary angiography* revealed markedly retarded flow in the lower lobes and middle lobe as well as narrowing of the arterial branches with decreased number of small branches. *Lung function tests* (table IV) showed severe obstructive ventilatory impairment without signs of reversibility There was no eosinophilia.

Diagnosis. Chronic bronchitis and emphysema.

Case 27 (II_2) A foundryman, born in 1908, brother of the patient in case 26. In the 1930 s he had symptoms of allergic rhinitis and since about 1950 recurrent bronchitis without dyspnea. During 1961 his condition became worse with increased dyspnea and severe attacks of asthma He was treated with large doses of steroids and sustained a compression fracture in the thoracal spine. In 1963 the TLC was 148 per cent of predicted and $FEV_{1.0}$ 1 1 l and LCI 11.0 PaO_2 was 64 mm and $PaCO_2$ 40 mm Hg. During the latter part of 1963 he had rapidly progressing symptoms of asthma and was treated with ACTH continuously He died in January 1964 Post mortem examination revealed typical changes of asthma in the bronchi which were

Case 24 A female, born in 1895 Her brother had died abroad at 54 years of age. The probable cause of death was emphysema. She had had dyspnea with insidious onset since 1940 and with a marked progression during influenza in 1946 She had only scanty mucoid phlegm occasionally and no predisposition to infections. She denied ever having had pneumonia. She is now unable to be outdoors because of her dyspnea Her weight has remained fairly constant at 60 kg *Lung function tests* (table IV) revealed severe obstructive impairment without changes after aminophylline. *Chest X ray* showed reduced vasculature to the lower lobes. There was no blood eosinophilia

Diagnosis Primary emphysema.

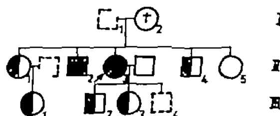


Family 19

Case 25

Case 25 (II_4) A female, born in 1901 Two siblings have died during infancy from pneumonia She had been treated with digitalis since 1955 because of mitral stenosis with auricular fibrillation Since 1958 she had had recurrent chest infections with more or less chronic cough with phlegm and increasing dyspnea but no signs of cardiac decompensation In 1963 cancer colli uteri was diagnosed and radiological treatment was started. In February 1964 she had pneumonia of the middle lobe. Since then her body temperature has been constantly elevated despite regression of the pneumonia *Lung function tests* showed obstructive ventilatory impairment and *chest X ray* showed marked arterial deficiency in the lower lobes. There was no eosinophilia She died from her cancer in June 1964 Post mortem examination revealed purulent bronchitis and emphysema with ruptured alveolar septa No paper mounted section was available in this case

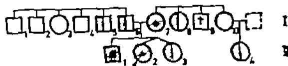
Diagnosis Chronic bronchitis and emphysema



Family 20

Case 26
Case 27

Case 26 (II_3) A female, born in 1911 Her brother (II_4, case 27) had chronic bronchitis. She had had recurrent chest infections with cough and phlegm since



Family 23

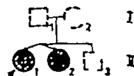
Case 30 (II_2) A female born in 1932. She had had intermittent uncharacteristic joint pains without any objective findings. She had a constantly elevated E.S.R and a serologic investigation revealed positive titers for listeriosis. She had no history of lung disease and the *chest X ray* and *pulmonary function tests* revealed nothing abnormal.

Case 31 (I_7) Female, born in 1903 the mother of the patient in case 30 She had always been in good health and had never had any lung disease. *Radiograms* and *lung function tests* were normal.

Case 32 (II_1) Male, born in 1928, the brother of the patient in case 30 He had no clinical signs of lung disease and was completely healthy *Radiograms* and *lung function tests* were normal.

Case 33 (I_{10}) A female, born in 1910 Except for leg ulcers she had always been in good health.

Comment Family 23 contains 4 members who are homozygotes for α_1-antitrypsin deficiency None of them had any signs of obstructive pulmonary disease.



Family 24

Case 34
Case 35

Case 34 (II_1) A female, born in 1934 In 1961 she had arthralgias, erythema nodosum and enlargement of the hilar glands. The condition was interpreted as sarcoidosis and it healed without special treatment. She had no clinical signs of obstructive pulmonary disease

Case 35 (II_2) Female, born in 1934, nonidentical twin of the patient in case 34 She had been treated in hospital in 1957 for rheumatic fever She has since been healthy

Comment Two nonidentical twins with homozygosity for α_1 antitrypsin deficiency None had any sign of obstructive pulmonary disease.

Additional cases (A_1—A)

These cases were found at the population survey in Värmland, 2 males and 2 females. So far they have only undergone a routine investigation and more definite data will be published later Hospital records were available in 2 cases.

Case A_1 A forester born in 1904 As a child he had scarlet fever with com

filled with masses of mucus with a considerable admixture of eosinophilic leucocytes The paper mounted section (fig 37) showed panacinar emphysema with marked deficiency of alveolar tissue

Diagnosis Asthmatic bronchitis and panacinar emphysema.

(Family 21) Case 28

Case 28 An elementary school teacher born in 1912 Her symptoms appeared about 1950 with increasing effort dyspnea during infection of the upper respiratory tract. Her main complaint has been dyspnea but in recent years she had increasingly frequent infections of the respiratory tract with symptoms of asthma. No precipitating factors apart from infections were known. *Lung function tests* (table IV) showed severe obstructive ventilatory impairment. *Chest X ray* showed reduced vasculature to the lower lobes and middle lobe There was no blood eosinophilia. She retired on a pension in 1960 because of her disease.

Diagnosis Probably a primary emphysema and asthmatic bronchitis.

(Family 22) Case 29



Fig. 38. Chest X ray in case 20 (1962)

Case 29 A female, born in 1917 In 1930 she was admitted to hospital because of a primary tuberculosis with an infiltrate in the left lung Her tuberculosis responded well to conservative treatment Since 1932 she has had repeated attacks of bronchitis with large amounts of purulent sputum. Already in 1936 chest x ray showed extensive peribronchitic changes. In 1958 bronchography showed bronchitic changes bilaterally and multiple grape sized bronchiectasis of the generalized type. No surgical treatment was possible. On account of increasing disability with recurrent chest infections and progressing effort dyspnea she retired on a pension in 1960 Her VC that year was determined as 1 4 l. Since 1958 she has a mild diabetes mellitus without complications. She had lost 6 kg in weight during the last five years. *Chest X ray* showed extensive fibrosis and in addition areas with deficient vasculature, suggesting emphysema (fig 38)

Diagnosis Chronic bronchitis, generalized bronchiectasis and emphysema

TABLE III *Survey of clinical histories, physical examinations, laboratory and radiological findings[a] in α_1-antitrypsin deficiency*

Case No.	Sex	Age	History: Heredity	History: Smoking	History: Peptic ulcer	Duration of symptoms (years): Phlegm	Duration of symptoms (years): Dyspnoea	Grade of dyspnoea	Heart failure	Examination: Rhonchi	Examination: Diminished breath sounds over bases	Examination: Clubbing	Electrocardiogram: RAH	Electrocardiogram: RVH	Blood: RBC	Blood: Eosinophilia	Chest X-ray: Emphysema degree	Chest X-ray: Group
1	M	64		●		30	15	III	o	o		o	●	●	4.8	+		III
2	F	56		●	o	6	5	III	●	o		●	●	●	4.3		++	II
3	M	62	●	o		12	10	II	●	●		o	o	o	4.8	o		III
4	M	60	+	o	●	10	15	III	o	●	●		o	●	4.9	●	++	III
5	M	58	o	o	●	15	15	IV		●	●		●	+	6.5	o	++	III
6	M	57	o			0	16	IV	o	o			o	o	4.2	●	++	II
7	M	56				32	20	IV	o		o	●	o	o	3.9			II
8	M	48	●	●	●	0	20	II	●	o		o	●	●	4.8	●		II
9	M	48	o	o	o	0	10	IV	●	o		o	●	●	3.1	o	+	II
10	M	46	o			10	3	IV		o		●			5.0	o		III
11	M	45	●		o	9	3	II	●	o	o	●	o	o	4.4	●		III
12	M	40	●		o	20	10	IV	●	●		o	o	●	4.3	o	+	II
13	M	39			o	9	5	III	●	●		o	●	●	4.9	o	++	II
14	F	40			●	8	11	II	●		o	o	●	o	4.4	●	+	II
16	M	38	+		●	6	6	III	●	●		o	●	o	5.0	●	++	III
19	M	25	●	●	o	0	0	I	●	o	●	●	o	o	5.3	o		III
22	F	40		●		5	30	IV	●	●		●	o	●	4.2	●	++	II
24	F	49		o	o	0	25	III	●	●	+	●	o	o	4.1	●		II
25	F	43	●	o	●	6	6	II	●	●	●	o	●	o	3.4	o	++	III
26	F	53		+	+	13	6	III	●	●		o	●	o	4.3	●		II
27	M	56		●	●	14	5	III	●	●	+	o	o	o	4.3			II
28	F	52	o		●	5	14	III	●	o		●	o	●	4.8	o	++	II
29	F	47	o	o	o	32	16	IV	●	●	o	●	o	o	4.3	o		IV

plicating otitis which resulted in loss of hearing He had always been doing heavy work in the forests and had no history or clinical evidence of obstructive lung disease.

Comment No clinical evidence of obstructive broncho-pulmonary disease.

Case A_2 A surveyor born in 1913 Since 1957 he had had progressive dyspnea without any symptoms of infections at all. At examination in hospital in 1962 and 1963 X ray revealed signs of advanced emphysema (degree + + group II) without definite evidence of bullae at supplementary tomography VC was 2 0 l (41 % pred) and $FEV_{1.0}$ 0 9 l (26 % pred.) without significant increase after inhalation of bronchodilatators

Diagnosis Primary emphysema

Case A_3 A female, born in 1907 Her paternal grandmother had had severe asthma Since 1935 the patient had had typical bronchial asthma with symptoms precipitated by a number of agents. At examination in hospital 1961 provocative tests were positive with *inter alia* dust timothy grass and deciduous tree pollen. During the last 10 years her disease has been dominated by dyspnea on exertion and intermittent attacks of asthma Roentgen-examination showed signs of emphysema (degree + group II) In 1960 her VC was 1 5 l and MVV 16 l/min There was no blood eosinophilia Blood specimens from the patient's relatives were studied See pedigree (family A_3) in fig 24 The patient's elder brother who was a homozygote, suffers from dyspnea on exertion but he has so far not been examined for the cause of this dyspnea.

Diagnosis Bronchial asthma (extrinsic type) and emphysema.

Case A_4 A woman born in 1917 She had for several years been troubled by recurrent infections of the respiratory tract but had little or no dyspnea on exertion. On the other hand the patient's brother (TIC not determined) had typical chronic bronchitis with increasing dyspnea on exertion None of these patients have been subjected to chest X ray or lung function tests.

Diagnosis Chronic bronchitis?

RESULTS

The clinical and laboratory findings are summarized in table III which also includes the roentgenographic findings Table IV gives a compilation of the results obtained in the lung function studies The results of special laboratory investigations are given in table V

The "hospital material consisted of 33 patients (17 were males and 16 females) with α_1 antitrypsin deficiency Definite evidence of chronic obstructive pulmonary disease was found in 23 of these patients Fifteen of these 23 patients were males and 8 were females If individuals below 40 years of age were excluded, it would mean that all males and two thirds of the females in this material had chronic obstructive pulmonary disease In 10 patients (2 males and 8 females) with α_1 antitrypsin deficiency (cases 18 20

TABLE III Survey of clinical history, physical examination, laboratory and radiological findings in α_1-antitrypsin deficiency

Case No	Sex	Age	History: Heredity	History: Smoking	History: Peptic ulcer	History: Duration of symptoms (years): Phlegm	History: Duration of symptoms (years): Dyspnoe	History: Grade of dyspnoe	History: Heart failure	Examination: Rhonci	Examination: Diminished breath sounds over bases	Examination: Clubbing	Electrocardiogram: RAH	Electrocardiogram: RVH	Blood: RBC	Blood: Eosinophilia	Chest X-ray: Emphysema degree	Chest X-ray: Group
1	M	61		●		30	15	III	o	o		o	o	●	4.8			III
2	F	50		●	●	0	5	III	o	●		o	o	●	4.3		++	II
3	M	62	o	o		12	10	II	●	●		o	o	o	4.8	o		III
4	M	50		o	●	10	15	III	●	o	o		●	o	4.9	o	++	III
5	M	52	o	o	o	15	15	IV		o	o		o	+	6.5	●	++	III
6	M	57	o			0	16	IV	o	o			o	o	4.6	●	++	II
7	M	58				22	20	IV	●		o	o	●	●	3.9		+	II
8	M	48	●	●	o	8	30	II	o	o		●	o	●	4.8	●		II
9	M	43	o	●	o	0	10	IV	o	●		o	●	o	5.1	o	++	II
10	M	46	●			10	8	IV		●		●			6.0	o	+	III
11	M	45	●		●	6	3	II	o	o	o	o	o	o	4.4	●	+	III
12	M	46	●		●	20	10	IV	●	●		o	●	o	4.6	o		II
13	M	39			●	8	3	III	o	o		●	o	●	4.9	o	++	II
14	F	40			●	5	11	II	●		●	o	●	o	4.4	●		II
15	M	38			●	6	8	III	●	●		o	o	●	3.6	o	++	III
16	M	25	o	●	o	0	8	I	●	o	●	o	●	o	3.3	●		III
22	F	49		●		2	30	IV	o	●		●	o	o	4.2	●	++	II
24	F	59		o	●	8	25	III	●	●		o	●	●	4.1	o		II
25	F	63	o	o	o	6	8	II	●	o	●	●	o	o	3.4	●	++	III
26	F	52				13	8	III	o	●		o	●	●	4.3	o		II
27	M	38		o	o	14	2	III	o	o		●	o	o	4.2			II
28	F	52	●		●	3	14	III	●	●		●	●	●	4.8	●	++	II
29	F	47	●	●	●	32	15	IV	●	●	●	o	o	o	4.3	o	+	IV

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Diagnosis Chronic bronchitis?

RESULTS

The clinical and laboratory findings are summarized in table III which also includes the roentgenographic findings Table IV gives a compilation of the results obtained in the lung function studies. The results of special laboratory investigations are given in table V

The hospital material consisted of 33 patients (17 were males and 16 females) with α_1 antitrypsin deficiency Definite evidence of chronic obstructive pulmonary disease was found in 23 of these patients Fifteen of these 23 patients were males and 8 were females If individuals below 40 years of age were excluded, it would mean that all males and two thirds of the females in this material had chronic obstructive pulmonary disease In 10 patients (2 males and 8 females) with α_1 antitrypsin deficiency (cases 18 20

TABLE V *Sodium in sweat bromsulphalein retention fibrinolytic activity and antiplasmin in some patients with α_1-antitrypsin deficiency*

Case No.	Sodium in sweat (meq/l)	Bromsulphalein retention	Fibrinolytic activity (mm² lysed area)		Antiplasmin
			citrated plasma	euglobulin prec.	
1	33	—	—	—	—
8	38	—	—	—	—
13	81	8	—	—	—
14	64	4	—	—	—
15	—	—	6	98	120
21	—	—	112	178	81
25	69	3	168	207	113
29	51	—	0	0	129
30	—	5	6	32	100

21 23, 30, 31 32, 33 34 and 35) clinical, radiographic or spirometric investigations revealed no certain signs of chronic obstructive pulmonary disease Six of these 10 patients were below 40 years.

A family history of chronic obstructive pulmonary disease was obtained in 11 cases. In 3 of the 12 sibships analyzed more than one sib was found to be affected two (case 1 and case 2) in family 1 three (cases 13 14 15) in family 13 and two siblings with chronic obstructive pulmonary disease in family 20 Of these, all except patient 15 (TIC not determined) also had α_1 antitrypsin deficiency In three families information was obtained about siblings who had died from pulmonary disease, but not about their α_1-antitrypsin level (families 3 17 18)

A family history of allergy was noted in only one patient (case 7) Special attention was given to the occurrence of pulmonary infections and allergic diseases during childhood. In case 2 there was a history of eczema of unknown type during childhood. In case 29 respiratory tract infections had started early but otherwise no evidence of any particular susceptibility to chest infections during childhood could be obtained. In no case had any asthmatic symptoms occurred during childhood. The approximate age at onset of the disease among the 23 patients with chronic obstructive pulmonary disease is given graphically in fig 39 About 60 per cent were below 40 at the onset of the disease and 90 per cent below 50 years

Eleven of the 23 patients gave a history of chronic cough and repeated respiratory tract infections before the onset of dyspnea. 9 patients had dyspnea as the primary symptom In only 2 patients was there a history of asthma before the onset of permanent dyspnea. In 8 patients there was a history of one or more attacks of pneumonia or other severe respiratory infection prior to development of more chronic chest illness. In 5 patients (cases 3 9 10 11 and 16) there was a history of acute chest illness resulting in permanent loss of exercise tolerance after recovery Of these cases 9 10 and 16 were classified as primary emphysema.

TABLE IV *Results of lung function tests*

Case No	$FEV_{1.0}$ (l)	$FEV_{1.0}$ % pred	$FEV_{1.0}$ aminophylline	FEV %	VC (l)	VC % pred.	FRC (l)	FRC % pred	TLC (l)	TLC % pred	LCI	PaO_2 mm Hg	$PaCO_2$ mm Hg
1	1.7	56	1.7	34	4.6	102	5.2	131	7.8	112	10.4	61	34
2	1.0	38	1.8	34	2.8	82	3.1	106	4.8	99	13.6	82	38
3	1.2	45	—	30	3.9	100	4.8	135	7.0	114	—	73	36
4	1.0	31	0.9	34	3.0	100	6.8	137	8.5	115	17.4	50	30
6	0.6	19	0.7	27	2.4	49	6.7	133	8.3	110	10.0	95	38
8	1.0	31	0.9	31	3.2	75	4.9	154	6.6	107	16.0	79	38
9	0.6	18	0.6	20	3.0	65	3.5	86	5.6	82	—	51	36
11	3.1	86	—	60	4.1	108	4.2	118	7.4	110	11.9	87	38
13	0.6	15	0.6	21	2.	87	5.8	150	7.7	116	—	81	37
14	0.4	38	1.0	26	3.4	111	3.7	158	5.9	138	15.1	81	34
16	0.5	19	—	21	3.8	70	6.0	143	8.4	112	12.0	82	33
19	2.6	53	2.7	78	3.3	84	5.0	117	6.7	88	11.7	85	34
22	0.9	51	0.8	38	2.3	98	3.5	197	5.3	148	16.5	84	33
24	0.7	34	0.8	37	2.0	88	4.0	158	5.0	108	—	—	—
3	1.2	52	—	44	2.5	80	3.1	111	4.8	106	—	64	34
26	1.1	46	1.0	38	2.7	88	4.0	186	6.5	141	—	66	43
28	0.8	34	—	37	2.2	73	2.5	108	4.1	9	—	74	35

bronchographic examination confirmed the suspicion of bronchiectasis. In case 11 and 29 there were generalized cystic bronchiectasis.

It is obvious from table IV and data given in the case reports that obstructive ventilatory impairment, as judged by the reduction in $FEV_{1.0}$ and FEV /s was present in all 23 patients with the possible exception of one (case 11) in whom these parameters were only slightly reduced. The mean percentage reduction in $FEV_{1.0}$ was 40 of the predicted value. In no patient except in case 2 was there any significant increase in $FEV_{1.0}$ after administration of aminophylline intravenously VC was normal or slightly reduced in all patients (mean 81 per cent of the predicted value) FRC was moderately increased in all patients (mean 137 per cent of predicted) as was TLC (mean 112 per cent of the predicted value) There were no significant differences in $FEV_{1.0}$, VC, FRC or TLC between those patients who had been assigned to roentgenographic group II and those in group III LCI was increased in most patients. Blood gas analysis revealed moderate to severe hypoxemia in several patients, but the mean reduction was slight to 75 mm Hg. In no patient in the whole material, except No 10 was there any hypercapnia (see case report) This patient had cor pulmonale at the time of determination. $PaCO_2$ in most patients fell below the normal range and the mean value calculated from table IV was 36 mm Hg

As mentioned in th introduction, the clinical diagnoses were based on anamnestic, clinical and laboratory data (including X ray) available at the time of the examination. Approximately half of the affected males and half of the affected females belonged to the group described as primary emphysema and the other half to the group described as chronic bronchitis (and/or bronchiectasis) and emphysema Extrinsic asthma was present in 2 cases. In three patients with primary emphysema there was evidence of earlier acute chest illness (cases 9 10 16)

All 110 family members studied with respect to total serum trypsin inhibitor capacity (see chapter V) were interwieved about the occurrence of earlier or existing pulmonary disease No physical examination, X ray studies or lung function tests were done. Among 64 individuals classified as heterozygotes, only 1 (case 17) had apparently had pulmonary disease. He is the brother of the patient in case 10 who had severe bullous emphysema. He had had a spontaneous pneumothorax but no signs of generalized obstructive pulmonary disease. Thoracoscopy revealed a few small emphysema blebs. Case 15 in family 12 is of interest in this connection. This patient obviously had emphysema. No blood sample was available in his case, but as he was married to an unaffected woman and had 2 children with normal TIC and 4 with intermediate TIC, he probably represented the heterozygous state of α_1-antitrypsin deficiency

None of th 22 relatives classified as normal with respect to TIC nor any of the spouses (13) were apparently

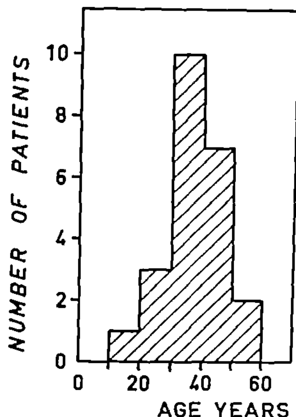


Fig. 39 Approximate age at onset of disease in 23 patients with α_1-antitrypsin deficiency

A history of radiographically verified peptic ulcers was noted in 7 of the 23 patients with chronic obstructive pulmonary disease and α_1 antitrypsin deficiency (table III) The ulceration was situated in the stomach in 1 (case 28) and in the duodenum in the remaining 6 One patient with α_1 antitrypsin deficiency but without chronic obstructive pulmonary disease (case 21) had had ulceration of the stomach.

Weight loss was common among the patients with chronic obstructive pulmonary disease (see case reports) In several there was severe weight loss despite restricted mobility because of the pulmonary disease (cases 2 6 9 10 13 26) None of the patients were overweight and most of them w... of the slender asthenic body buil... Hypertension with elevation of th... diastolic blood pressure was not not... in any of the cases. General physica... examination revealed diminishe... breath sounds over the bases in most of the patients. Rhonci were audible in 2 patients at the time of investigation (table III) Both barrel chest and diminished heart sounds were common, but were not used as signs of emphysema. The electrocardiogram revealed right ventricular hypertrophy in only 2 cases (5 and 10) Both had had heart failure. Polycythemia was found in only 1 patient (case 5) Blood eosinophilia was demonstrated in 4 patients (table III) Liver function was studied by bromsulphalein retention in 4 cases and was found to be normal in all (table V) The sodium content of the sweat was within the normal range in all 6 patients studied (table V) Tests for fibrinolytic activity were performed in 5 patients it was normal in three patients slightly increased in case 25 and markedly increased in case 26 Antiplasmin was found to be normal in all 5 patients (table V)

The roentgen findings are summarized in table III Signs of emphysema were found in all 23 patients severe (++) in 10 and moderate (+) in 13 When the material was classified according to Scarrow (1964) most patients (13) fell into group II (absence of inflammatory changes) and 9 into group III (moderate inflammatory changes) In case 29 the roentgenograms were dominated by inflammatory changes (group IV) and the

As the diagnosis of chronic bronchitis seems to be made by fairly uniform criteria in official Scandinavian statistics, the prevalence of chronic bronchitis in the Scandinavian countries can be expected to be roughly the same. Bentsen (1962) investigated the prevalence of asthma bronchitis-emphysema in a Norwegian agricultural district with 5 000 inhabitants. He found a frequency in this population of 1 19 per cent. The prevalence was much higher 3 14 per cent, when only the age group 50— 0 years was considered. A similar study has been performed in Rönne Bornholm, Denmark, by Olsen and Gilson (1960) They studied the prevalence of hronic bronchitis in a representative sample of men, aged 55—64 and found a frequency of 2.6 per cent for this disease. The figures of Bentsen and those of Olsen nd Gilson give an approximative frequency of chronic obstructive pulmonary disease of 2—3 per cent in the higher age groups. Tibblin and Wilhelmsson (1965) investigated the incidence of chronic bronchitis in Gothenburg, Sweden. Their material consisted of 855 men of 50 years of age. They found a frequency of 2.5 per cent The frequency in Sweden is thus of the same order of magnitude as in Denmark and Norway This means that roughly every 30th person above 50 in Sweden should be affected by such disease. This figure gives no idea of the frequencies of for example, primary emphysema compared with that of chronic bronchitis and emphysema. If the frequency of homozygotes for α antitrypsin deficiency in a Swedish population is taken as 1 1700 and the frequency of chronic obstructive pulmonary disease as 1 30 the probability of these two conditions occurring in the same person by chance is $\frac{1}{30} \times \frac{1}{1700}$ or 0 00002 In an unselected material of 7,000 individuals one should thus expect 7,000×0.00002 = =0 14 individuals to have both conditions. The real incidence was 2. The last mentioned figure is provisional owing to the smallness of the number of homozygotes found in the population survey but, on the other hand they represent a large sample. The figures presented strongly support the assumption that the incidence of chronic obstructive broncho-pulmonary disease is higher in persons homozygous for α_1-antitrypsin deficiency than in the general population.

So far no definite figures can be gi en for the frequency of chronic obstructive pulmonary disease among individuals with α_1 antitrypsin deficiency The relative overall frequency in the hospital material was 0 7 As this figure is based on a selected material, it might be too high. The relative frequency in the unselected material (A_1—A_4) was 0.5 This figure is uncertain on account of the smallness of the material. Assuming the true frequency to be 0.5 the incidence of chronic obstructive broncho-pulmonary disease among homozygotes would be approximately 15 times higher than in the general population It would therefore appear justified to assume the existence of an association between α_1-antitrypsin deficiency and chronic obstruc

affected by chronic obstructive lung disease.

In three children with cystic fibrosis of the pancreas a normal or slightly elevated TIC was found.

DISCUSSION

The high frequency of chronic obstructive broncho-pulmonary disease found in the patients with α_1 antitrypsin deficiency cannot be ascribed to chance. Of the 33 hospital patients, as many as two thirds showed clear evidence of the disease. It might be objected that this material is selected because of all the patients had been admitted to hospital and that investigation there had included paper electrophoresis, which had revealed the α_1 antitrypsin deficiency There is no reason to suppose that investigation of this type of pulmonary disease includes electrophoresis more often than of other diseases. In none of the cases had the patients been referred for electrophoresis for the occurrence of α_1 antitrypsin deficiency The patients had been referred for examination mainly because of increased erythrocyte sedimentation rate or recurrent infections, i e a finding suggesting the possibility of hypo-γ globulinaemia In many cases the examination was included as a routine procedure in the investigation of the patients on admission to hospital. Healthy persons with α_1 antitrypsin deficiency are, of course under represented in the present material but then again there is no reason to suppose that persons with α_1 antitrypsin deficiency are not attacked just as often as other persons by such diseases with associated increased erythrocyte sedimentation rate indicating paper electrophoretic examination of the serum proteins A more accurate estimation of the incidence of obstructive pulmonary disease in patients with α_1 antitrypsin deficiency can be obtained from examination of the 6 995 subjects reported in chapter VI Of those, 4 (additional cases) were found to be homozygous for α_1-antitrypsin deficiency Two (A_2 and A_3) of them had clear cut evidence of obstructive pulmonary disease one (A_2) had severe primary emphysema while the clinical picture of the other (A_3) was primarily dominated by asthmatic symptoms but now by ventilatory insufficiency because of roentgenologically verified emphysema In a third case (A_4) there was evidence of chronic bronchitis but not strong enough to allow a firm diagnosis of chronic obstructive pulmonary disease The frequency of homozygosity for α_1 antitrypsin deficiency had been previously calculated as about 1 1700 (chapter VI) No reliable figures are available on the frequency of chronic obstructive pulmonary disease in a Swedish population The approximate frequency could however be calculated indirectly in the following way

In a compilation of the death rates of chronic bronchitis per 1 000 inhabitants in 21 European countries L FT tore (1951) found 0 06 for Denmark 0 08 for Norway and 0 05 for Sweden

As the diagnosis of chronic bronchitis seems to be made by fairly uniform criteria in official Scandinavian statistics, the prevalence of chronic bronchitis in the Scandinavian countries can be expected to be roughly the same. Bentsen (1962) investigated the prevalence of asthma-bronchitis-emphysema in a Norwegian agricultural district with 5,000 inhabitants. He found a frequency in this population of 1 19 per cent. The prevalence was much higher 3 14 per cent, when only the age group 60—70 years was considered. A similar study has been performed in Rönne, Bornholm, Denmark, by Olsen and Gilson (1960) They studied the prevalence of chronic bronchitis in a representative sample of men, aged 55—64 and found a frequency of 2.6 per cent for this disease. The figures of Bentsen and those of Olsen and Gilson give an approximative frequency of chronic obstructive pulmonary disease of 2—3 per cent in the higher age groups. Tibblin and Wilhelmsson (1965) investigated the incidence of chronic bronchitis in Gothenburg, Sweden. Thei material consisted of 85ᴗ men of 50 years of age. They found a frequency of 2.5 per cent. The frequency in Sweden is thus of the same order of magnitude as in Denmark and Norway Ti is means that roughly every 30th person above 50 in Sweden should be affected by such disease. This figure gives no idea of the frequencies of for example, primary emphysema compared with that of chronic bronchitis and emphysema. If the frequency of homozygotes for α antitrypsin deficiency in a Swedish population is taken as 1 1700 and the frequency of chronic obstructive pulmonary disease as 1 30 the probability of these two conditions occurring in the same person by chance is $\frac{1}{30} \times \frac{1}{1700}$ or 0 00002. In an unselected material of 7 000 individuals one should thus expect 7,000 × 0.00002 = = 0 14 individuals to have both conditions. The real incidence was 2. The last mentioned figure is provisional owing to the smallness of the number of homozygotes found in the population survey but on the other hand, they represent a large sample. The figures presented strongly support the assumption that the incidence of chronic obstructive broncho-pulmonary disease is higher in persons homozygous for α_1 antitrypsin deficiency than in the general population.

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tive pulmonary disease Whether this association means a pathogenetic relationship between the dysproteinemia and the lung disease cannot yet be proved but is more fully discussed in chapter VIII

With the above mentioned figures for the frequencies of α_1 antitrypsin deficiency and chronic obstructive pulmonary disease in a Swedish population one can also calculate the expected incidence of α_1 antitrypsin deficiency *among patients with obstructive pulmonary disease.* Assuming a 100 per cent clinical manifestation among homozygotes, the maximal incidence of α_1 antitrypsin deficiency among such patients would be $\frac{30}{1700}=0\ 018$ or 2 per cent. This figure probably means an overestimation since not all patients with α_1 antitrypsin deficiency have pulmonary disease. The real incidence is probably lower or about 1 per cent. This figure is in agreement with the findings of Kueppers, Briscoe and Bearn (1965) who found one case with α_1 antitrypsin deficiency among 99 random patients attending an emphysema clinic. Preliminary studies on the frequency of homozygotes among patients with obstructive ventilatory impairment and attending the Department of Clinical Physiology at this hospital for spirometric studies have given figures of the same order of magnitude.

Of the 64 individuals classified by their TIC level as heterozygotes in quiry into the personal histories revealed pulmonary disease in only one (case 17) He had a local bullous emphysema, but no signs of generalized obstructive disease. Judging from the recessive heredity of α_1 antitrypsin deficiency patient 15 in family 12 was a heterozygote. He had roentgenological signs of emphysema. In none of the relatives with a normal TIC could any such disease be demonstrated. In the present material, then, no increase in the frequency of chronic obstructive pulmonary disease could be demonstrated with certainty among relatives *with a normal or intermediate TIC* level. On the other hand, the material did include examples of familial obstructive pulmonary disease (families 1 12 and 20) The combination of α_1 antitrypsin deficiency and pulmonary disease in several members of a given family lends further support to the postulated connection between these two conditions.

The term obstructive broncho-pulmonary disease includes various more or less distinguishable entities, namely asthma bronchiale, chronic bronchitis and emphysema. Only recently has there been general agreement that emphysema should be defined in anatomic terms and that emphysema can be diagnosed and classified consistently *only on post mortem* preparations from lungs distended and fixed before they are cut (WHO Report of an expert committee 1961) In the present work emphysema was defined in anatomical terms. Since only few post mortem specimens were available the diagnosis of emphysema was based on the anamnestic, clinical, radiological and physiological data In patients where *unremitting exertional dyspnea is the*

dominating symptom and the lung function tests are compatible with mainly irreversible obstructive ventilatory impairment the probability for the existence of anatomic emphysema is high. In such cases the radiographs offer a possibility to estimate the degree of loss of tissue substance. Laws and Heard (1962) found a close correlation between anatomic emphysema detected after death and radiographic findings *intra vitam* and pointed out that the reduction in the vascular bed was the most reliable sign of anatomic emphysema. Tomography of the entire lung or selective angiography can demonstrate these changes more precisely (Bates and Christie, 1964). In the present work the radiographic evaluation f the presence and severity of emphysema was based on the changes in the peripheral vascular shadows. Generalized radiolucency a flat diaphragm and increased retrosternal space were considered less reliable signs of anatomic emphysema since they may merely reflect a hyperinflation of reversible character as in an acute attack of asthma

The term primary emphysema has been used here to describe emphysema not antedated by chronic bronchitis. Dyspnea is the dominating primary symptom in this group of patients, and they d not have a history of repeated pulmonary infections. The possibility that they have clinically silent bronchitis cannot be excluded.

The diagnosis of chronic bronchitis is based on the clinical history and in most patients no anatomic correlate is a ailable except bronchitic changes sometimes revealed at bronchography The criteria used in the diagnosis of chronic bronchitis are unsatisfactory because they are based on the patient s personal opinion of the severity of cough and of the amount of phlegm brought up. It is known that these patients tend to belittle chronic and often severe cough (Mitchell and Filley 1964)

It is also difficult to differentiate between asthma and chronic bronchitis with superimposed bronchospastic symptoms. Two types of asthma are recognized. The extrinsic type usually appears before the age of 20 and is often accompanied l y demonstrable allergy in the patient and in his family Skin tests are frequently positive. This type of asthma usually runs a long course and is seldom associated with emphysema (Gough, 1961) The intrinsic type often develops after the age of 30 It is characterized by more or less chronic bronchospasm and excessive mucoid sputum, both of which are aggravated by respiratory tract infections, air pollution and adverse weather The skin tests are often negative and the prognosis is often poor (Oswald, 1959) These patients are often regarded as asthmatic in the United States and as bronchitic in Britain. The term asthmatic bronchitis has been used here to describe this type of disease.

Correct classification of the various entities covered by the blanket term chronic obstructive broncho-pulmonary disease is obviously difficult Neither will extensive lung function studies distinguish with certainty be

tive pulmonary disease. Whether this association means a pathogenetic relationship between the dysproteinemia and the lung disease cannot yet be proved, but is more fully discussed in chapter VIII

With the above mentioned figures for the frequencies of α_1 antitrypsin deficiency and chronic obstructive pulmonary disease in a Swedish population one can also calculate the expected incidence of α_1 antitrypsin deficiency among patients with obstructive pulmonary disease. Assuming a 100 per cent clinical manifestation among homozygotes the maximal incidence of α_1 antitrypsin deficiency among such patients would be $\frac{30}{1700} = 0.018$ or 2 per cent. This figure probably means an overestimation since not all patients with α_1 antitrypsin deficiency have pulmonary disease. The real incidence is probably lower or about 1 per cent This figure is in agreement with the findings of Kueppers Briscoe and Bearn (1965) who found one case with α_1 antitrypsin deficiency among 99 random patients attending an emphysema clinic. Preliminary studies on the frequency of homozygotes among patients with obstructive ventilatory impairment and attending the Department of Clinical Physiology at this hospital for spirometric studies have given figures of the same order of magnitude.

Of the 64 individuals classified by their TIC level as heterozygotes, inquiry into the personal histories revealed pulmonary disease in only one (case 17) He had a local bullous emphysema, but no signs of generalized obstructive disease. Judging from the recessive heredity of α_1 antitrypsin deficiency patient 15 in family 12 was a heterozygote. He had roentgenological signs of emphysema. In none of the relatives with a normal TIC could any such disease be demonstrated. In the present material, then, no increase in the frequency of chronic obstructive pulmonary disease could be demonstrated with certainty among relatives with a normal or intermediate TIC level. On the other hand, the material did include examples of familial obstructive pulmonary disease (families 1 12 and 20) The combination of α_1 antitrypsin deficiency and pulmonary disease in several members of a given family lends further support to the postulated connection between these two conditions

The term obstructive broncho-pulmonary disease includes various more or less distinguishable entities, namely asthma bronchiale, chronic bronchitis and emphysema Only recently has there been general agreement that emphysema should be defined in anatomic terms and that emphysema can be diagnosed and classified consistently only on post mortem preparations from lungs distended and fixed before they are cut (WHO Report of an expert committee, 1961) In the present work emphysema was defined in anatomical terms. Since only few post mortem specimens were available the diagnosis of emphysema was based on the anamnestic, clinical radiological and physiological data In patients where unremitting exertional dyspnea is the

from table III that heart failure was uncommon. Frank cor pulmonale was present in only 2 patients (cases 5 and 10). Patient No. 5 had a long history of repeated respiratory tract infections, the radiographs showed extensive inflammatory changes, and he was the only patient in the material who had a polycythemia. Terminally he developed cor pulmonale. He had no hypercapnia and bronchiectasis were found at autopsy (see case report). In only one patient (case 10) who also had cor pulmonale, there was evidence of alveolar hypoventilation. His clinical history was not dominated by infections, the radiographs showed only slight inflammatory changes, and he had no polycythemia. He is probably an example of the type of patients with a primary emphysema who terminally develop cor pulmonale. Patient No. 29 showed extensive inflammatory changes in the pulmonary parenchyma (fig. 88) of the type described as honey-comb lung. She also had generalized bronchiectasis. Data on blood gas tensions are lacking in this patient, but so far she has had no heart failure and the electrocardiograms have shown no evidence of right ventricular hypertrophy. Electrocardiography showed no signs of right ventricular hypertrophy in any of the patients except Nos. 5 and 10. This does not, of course, exclude the presence of such hypertrophy especially as leads V_3R or V_4R have not been used in this series of patients.

According to Burrows, Niden, Fletcher and Jones (1964) patients with chronic obstructive pulmonary disease can be classified as type A (alveolar or emphysematous type), type B (bronchitic or inflammatory type) or type X (indeterminate type). Their series consisted of 100 patients and their primary classification was based on the roentgenologic findings. Type A patients often showed roentgenologic emphysema, elevated TLC, but rarely chronic hypercapnia or evidence of cor pulmonale. Type B patients, on the other hand, often show roentgenologic evidence of inflammatory disease. TLC is usually within normal limits, and hypercapnia and cor pulmonale are common. They also suggested that most type A patients have severe generalized panacinar emphysema, while type B patients *may* have little or no emphysema at autopsy.

As to the spirometric parameters ($FEV_{1.0}$, VC, TLC or FRC), RBC, PaO_2 or $PaCO_2$ group II did not differ significantly from group III. This may be explained in different ways: the material was small and therefore significant trends, if any, may remain concealed. In the present work the patients were assigned to roentgenologic group III even when only slight inflammatory changes were seen. Three cases (Nos. 4, 16, 25) in this group also had signs of pronounced emphysema, and many of these patients may be comparable to Burrow's et al. type X and thereby minimize any true differences between type A and type B patients.

Roentgenological evidence of emphysema was seen in all 23 patients. In case 11 and case 19 emphysema

tween anatomic emphysema and chronic bronchitis without any substantial degree of emphysema. Therefore, in the diagnosis of chronic obstructive broncho-pulmonary disease all clinical findings, roentgenologic signs and the results of lung function tests must be taken into account.

After classification of the patients according to the above mentioned criteria α_1 antitrypsin deficiency was not found to be associated with any uniform type of chronic obstructive broncho pulmonary disease Though the material was small and included various types of obstructive pulmonary disease certain conclusions appeared warranted.

The age of onset of disease was remarkably low (fig 39) About sixty per cent were below 40 years of age at the onset of disease and 90 per cent below 50 years. No epidemiologic studies on chronic obstructive broncho-pulmonary disease in a Swedish population are available for comparison but there seems to be general agreement that pulmonary emphysema is only occasionally seen below the age of 40 years (Ebert and Pierce, 1963) The peak incidence of pulmonary emphysema is reached in the 6th and 7th decades (Thurlbeck, 1963 a) The present material included several young patients severely disabled by their pulmonary disease (cases 9 10 11 12, 13 16) Obstructive pulmonary disease was more common in the males than in the females. When individuals below 40 years of age were excluded the incidence was 100 per cent in the males, against 67 per cent in the females This difference is not statistically significant and the material was too small to allow further conclusions about the sex distribution of chronic obstructive pulmonary disease in α_1 antitrypsin deficiency It may however be mentioned that males are predominant in most large compilations of cases of emphysema and/or chronic bronchitis (For a review see Thurlbeck, 1963 a)

The frequency of primary emphysema in the present material was strikingly high Judging from anamnestic data half of the males and half of the females had this condition. These patients had effort dyspnea as the primary symptom and dyspnea dominated the clinical picture also later They often had no history of infections, and roentgenography showed signs of severe emphysema but only slight or no signs of inflammation From a functional point of view these cases are characterized by severe ventilatory insufficiency with only moderate hypoxaemia and normal or slightly lowered CO_2-tension Typical examples of this variant are cases 6, 8, 9 13 16 22 and 24 This type of patients is called emphysema with hyperventilation (WHO report of an expert committee, 1961) These patients rarely have cardiac complications and they develop cor pulmonale only terminally or with the onset of severe acute respiratory tract infections. They differ in their clinical course from those with the syndrome of alveolar hypoventilation where hypercapnia polycythemia and cor pulmonale are more common It is obvious

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was only slight. Case 11 is of special interest as the bronchography (fig 34) showed a peculiar type of generalized small-cystic bronchiectasis. The bronchiectatic alterations resembled the type described by Loeschke (1928) and Israëls (1962) A similar type of bronchiectasis was found in case 29 in which the radiographs were however dominated by extensive fibrosis Case 11 occupies a particular position in the material not only because of the special bronchiectasis but also because of the mildness of the obstructive ventilatory impairment In case 19 evaluation of the peripheral vessels was difficult owing to the occurence of a fine nodular fibrosis in the lung parenchyma

Since no post mortem specimens were available except in case 27 in which the paper mounted section showed panacinar emphysema the cases could not be classified according to type of emphysema The existence of two distinctive types of emphysema the centrilobular and panacinar types, is now generally accepted. (For a review see Thurlbeck, 1963 a) These two types often occur together at least when the emphysema is severe Thurlbeck (1963 b) found the centrilobular type to involve the upper zones more frequently and more severely He found the panacinar emphysema more or less evenly distributed spatially in the lungs, but in cases of severe emphysema panacinar emphysema involved the lower zones of lung more frequently and more severely In the present material the emphysema as judged from the radiographs, was invariably more pronounced in the lower zones This is most clearly seen in those cases where pulmonary angiography have been performed (case 1 fig 29 and case 9 fig 33) This finding is, of course not proof that we are dealing with a panacinar emphysema but it is impressive, especially since most of the type A patients of Burrows et al. (1964) were believed to have panacinar emphysema.

Asthma of the extrinsic type was uncommon. The patient in case 2 undoubtedly had an extrinsic asthma but in addition the history radiographs and lung function tests revealed evidence of emphysema. A similar situation was found in case A_2. Absence of eosinophilia (table III) also supports the view that true allergic asthma is uncommon among these patients. On the other hand, broncho-spastic symptoms were common but this does not mean that the patients have asthma in the strict sense of the word, but merely an increased bronchial reactivity to different agents. The finding of an unchanged $FEV_{1.0}$ after administration of a bronchodilatatory drug (table IV) supports the concept of irreversible air way obstruction but cannot be taken as proof of anatomic emphysema On the other hand a significant increase in $FEV_{1.0}$ after treatment with aminophylline is clear evidence that the obstructive impairment is due in part to reversible bronchospasm.

Judging from the clinical and roentgenological findings together with the results of the lung function tests, a typical case of chronic obstructive broncho-pulmonary disease with associated α_1 antitrypsin deficiency may be de-

cribed as follows A male, below 40 years of age, without a previous history of chronic cough and phlegm, who develops a progressive effort dyspnea due to widespread roentgenographic emphysema, predominantly in the lower zone and without radiographically demonstrable severe inflammatory changes. Lung function tests show a severe ventilatory insufficiency of the obstructive type but with only slightly affected blood gas tensions suggesting disturbed ventilation perfusion ratio but without signs of alveolar hypoventilation. Only terminally or at onset of severe respiratory infections is there cor pulmonale, and polycythemia is absent. The presence of similar disease in relatives, especially siblings, increases the chances of coexisting α antitrypsin deficiency This type of patient resembles the group E patients of Fletcher et al. (1963) and the type A patient of Burrows et al. (1964)

The frequency of primary emphysema, relative to that of chronic bronchitis and emphysema in a Swedish population is unknown. Judging from Bentsen s figures (1962) it is relatively low in Norwegian population and possibly the same holds true also for a Swedish population. Patients with extreme dyspnea normal blood gases, and severe emphysema seem to be more common in the United States (Richards, 1960) while they are relatively uncommon in Britain (Fletcher Hugh Jones, McNicol and Pride, 1963) The frequency of α_1-antitrypsin deficiency in chronic obstructive broncho-pulmonary disease would be much higher than the calculated 1 per cent if a group of patients with primary emphysema were selected. Seebohm and Bedell (1963) have published a series of 10 such patients with emphysema appearing early in life, but they give no electrophoretic data.

The clinical syndrome of emphysema has long been thought to have a familial tendency (for a review see Wimpfheimer and Schneider 1961) Obviously α_1-antitrypsin deficiency might be an associated condition in families where several members are known to have emphysema, but, so far no electrophoretic data have been given in reports of such families.

An increased *frequency of peptic ulcers*, especially duodenal ulcers, in patients with chronic obstructive broncho-pulmonary disease has been found by many authors (for a review see Hegetschweiler Hunziker and Marantz, 1960) In the present series the frequency of peptic ulcers, mostly duodenal ulcers, was as high as 30 per cent. The same figure was obtained by Mitchell and Filley (1964) in a series of 160 patients with chronic obstructive broncho-pulmonary disease. No definite explanation of this association has so far been offered. There is no reason to believe that α -antitrypsin deficiency *per se* predisposes to peptic ulcers.

It is generally accepted that the *sodium content of sweat* in children with fibrocystic disease of the pancreas is much larger than that in normal children. It has also been claimed that a somewhat raised electrolyte content of sweat occurs in parents and siblings of such children and that this indicates

the heterozygous state of the disease (Smoller and Hsia, 1959) It has also been stated that some adult patients with chronic chest disease, such as chronic obstructive pulmonary emphysema, show raised levels and should be considered "formes frustes" of fibrocystic disease of the pancreas (Wood, Fishman Reemtsma Barker and Di Sant Agnese, 1959) The results obtained by other workers (Anderson and Freeman 1960) provide no evidence that the carrier state in fibrocystic disease can be determined from raised levels of sweat sodium. The sweat sodium level was determined in 6 patients with α_1 antitrypsin deficiency (table V) with normal result in all. Furthermore the α_1 antitrypsin level was found to be normal in 3 children with classic fibrocystic disease of the pancreas and in none of the families with α_1 antitrypsin deficiency was there any known case of fibrocystic disease. These findings warrant the conclusion that α_1 antitrypsin deficiency and fibro-cystic disease of the pancreas are two completely unrelated conditions.

The site of synthesis of α_1 antitrypsin is unknown but it might be the liver since a low α_1 antitrypsin level can be seen in association with the reduction of the albumin in advanced cirrhosis of the liver (see chapter V) As the paper electrophoretic pattern in α_1 antitrypsin deficiency shows no gross abnormalities except lack of the α_1 band advanced liver parenchymal damage in these patients can *a priori* be excluded. The bromsulphalein retention was normal in 4 patients (table V) studied. Thus, the low α_1 antitrypsin level in the deficiency state cannot be ascribed to acquired liver damage.

The results of the fibrinolytic tests given in table V are of interest in view of the demonstration that destruction of the protease inhibitor capacity of serum with chloroform (Christensen, 1946) or acetone (Astrup 1956) is followed by an activation of the fibrinolytic system. From table V it is obvious that α_1 antitrypsin deficiency *per se* is not necessarily associated with increased fibrinolytic activity Three of the 5 patients studied showed normal fibrinolytic activity In case 25 the increased fibrinolytic activity might be ascribed to the presence of an advanced genital cancer (see case report) In case 26 the cause of the increased fibrinolytic activity remains obscure. Hypoxemia may be a factor of importance. In all cases the fibrinogen level was normal.

Two types of antiplasmin are found in human serum one is associated with the α_1 globulin fraction and one with the α_2-globulin fraction (Norman and Hill 1958) The α_2-antiplasmin is probably identical with α_2-antitrypsin (Jacobsson, 1955) It was found (chapter IV) that the α_2-antitrypsin activity remains unchanged in α_1 antitrypsin deficiency a finding supported further by a normal antiplasmin activity in 5 patients with α_1 antitrypsin deficiency (table V)

SUMMARY

Of 33 hospital patients who were homozygous for α_1 antitrypsin deficiency 23 had chronic obstructive broncho-pulmonary disease. Of the patients above 40 years, all the males and two thirds of the females had the disease.

Of four patients with this dysproteinemia detected at a population survey of 7 000 individuals, definite evidence of chronic obstructive broncho-pulmonary disease was found in two.

An association was found between α_1-antitrypsin deficiency in its homozygous form and chronic obstructive broncho-pulmonary disease. The incidence of such pulmonary disease in α_1-antitrypsin deficieny was at least 15 times higher than in the Swedish population as a whole. α_1-antitrypsin deficiency can be expected in only 1 per cent of individuals with chronic obstructive broncho-pulmonary disease

The material included three families with more than one member with both α_1 antitrypsin deficiency and chronic obstructive broncho-pulmonary disease. No increased incidence of the disease was found among relatives who had a normal level of $\alpha_1$antitrypsin or who were heterozygous for the deficiency

No special type of chronic obstructive broncho-pulmonary disease is associated with α_1 antitrypsin deficiency In most of the patients the onset of the disease was early (below 40 years of age) Roentgenological signs of emphysema, always of the lower zone, was noted in all 23 patients. Clinically about half of the cases could be classified as primary emphysema. Severe ventilatory insufficiency was common. Alveolar hypoventilation, cor pulmonale, and polycythemia were rare.

Cystic fibrosis of the pancreas and α_1-antitrypsin deficiency are *two* unrelated conditions.

No evidence of acquired liver damage or increased fibrinolytic activity in blood could be found among patients with α_1-antitrypsin deficiency

Chapter VIII

GENERAL DISCUSSION

An association was found between α_1 antitrypsin deficiency and the occurrence of chronic obstructive broncho pulmonary disease (chapter VII) With the possible exception of cystic fibrosis of the pancreas no biochemical abnormality has ever before been found in this multifacetted group of diseases. α_1 antitrypsin deficiency was found to be a biochemical defect transmitted by a recessive gene. The occurrence of the mutant gene in a single dose was accompanied by an intermediary manifestation (*heterozygotes*) while the gene in a double dose gave rise to a full homozygous manifestation with reduction of α_1 antitrypsin activity to about 12 per cent of normal (chapter V) Only the homozygous condition produced clinical manifestations in the form of pulmonary disease while the heterozygous form produced no demonstrable disorder

All clinical forms of chronic obstructive broncho pulmonary disease were represented in the present material (chapter VII) An invariable feature was radiological signs of emphysema Primary emphysema was strikingly common, but it was difficult to decide with certainty whether the emphysema in the individual cases was due to bronchitis pneumonia or perhaps a subclinical pulmonary infection.

The aetiology of emphysema is not properly understood and there is no unitary hypothesis for the pathogenesis of this disease (Richards, 1960)

Whether anatomical emphysema is caused primarily by inflammatory (bronchiolitis) or obstructive changes the loss of tissue substance, which is basic to all forms of emphysema, must be explained (Wright and Kleinerman 1963) Increased proteolytic activity *might well be responsible for the loss* of tissue substance, and it appears reasonable to assume that a genetic deficiency characterized by lack of an antiprotease might be accompanied by an excessive proteolytic activity with consequent damage to the lung

Determination of the α_1 antitrypsin level in the lung tissue and in bronchial mucus in individuals affected with α_1 antitrypsin deficiency compared with that in normals might yield further information on the inhibitory effect of α_1 antitrypsin on the proteolytic enzymes occurring in the lung parenchyma.

A question that then unsought arises is what proteolytic ferments might be of significance in this respect Bacteria

or viruses may of course, be of importance, but is sounds more likely to assume liberation of ferments from endogenous cells. The leucocytes, which are known to contain leucoproteinases that are inhibited by serum antiprotease (Grob, 1949) are one possible source. Wright and Kleinerman (1963) emphasize the peribronchiolar accumulation of macrophages which are known to contain lysing enzymes.

The alveolar macrophages are known effectively to phagocytose inhaled bacteria (Green and Kass, 1964) and they too, may be a source of proteolytic enzymes. A wide variety of endogenous cells may be potent sources of proteolytic ferments. One might very well imagine that a continous release of proteolytic ferments occurs in association with the death of cells, especially of cells with a high turnover rate. Normally such ferments are inactivated inhibitory substances, for example α antitrypsin.

Various exogenous factors in the form of infections or other noxious agents may temporarily disturb this equilibrium with excessive proteolytic activity as a result. Such a disturbance of the equilibrium might be considered in α_1-antitrypsin deficiency.

Investigations of the occurrence of proteolytic ferments in different cell populations in the lung parenchyma and the inhibitory effect of α_1 antitrypsin on the ferments would perhaps produce further evidence in support of the mechanism outlined above. Also *in vitro* studies of the resistance of the lung tissue to various proteolytic ferments would probably be informative.

Despite the absence of any obvious lowered resistance to infection in many of the patients with signs of severe emphysema (chapter VII) one might assume that the increased proteolytic activity with consequent dissolution of lung tissue and emphysema is triggered off by infections, perhaps also by subclinical infections. A prospective investigation of these patients with radioactive isotopes to chart the regional ventilation and perfusion might elucidate the course of the morbid anatomic conditions in the lung parenchyma, particularly in association with infections.

As in other genetic entities, the degree of clinical manifestation in α_1 antitrypsin deficiency is highly variable, ranging from complete absence of clinical symptoms to an early onset of severe emphysema with pronounced disability. In those with pulmonary disease the clinical picture was variable but true to type in the sense that obstructive ventilatory insufficiency and radiological emphysema were almost invariably present. It is not known why certain homozygotes for α_1-antitrypsin deficiency do not develop obstructive pulmonary disease. The incomplete penetrance of the clinical manifestation in some persons may indicate lack of exposure to some hitherto unknown environmental factor. On the other hand, the expressivity of a gene also depends on its interaction with other genes. Family 23 (chapter VII) included four members with α_1-antitrypsin deficiency but

without signs of pulmonary disease. Modifying genes may be responsible for the resistance to obstructive broncho-pulmonary disease in such a family So far no evidence has been produced suggesting genetic hetero geneity of α_1 antitrypsin deficiency

The observations and considerations set forth above appear to warrant the tentative conclusion that hereditary α_1 antitrypsin deficiency predisposes to the development of obstructive broncho-pulmonary disease, especially emphy sema, owing to an impaired resistance of the lung parenchyma to proteolytic enzymes possibly liberated in associa tion with infections

GENERAL SUMMARY

A method was described for determining serum trypsin inhibitor capacity (TIC) using benzoyl arginine p-nitroanilide as a substrate for trypsin. The mean TIC in 100 normal individuals was 1.07±0.12 (S.D.) mg trypsin inhibited per ml serum.

Separation in agar gel demonstrated two main trypsin inhibitors in human serum. The main activity or about 90 per cent was found in the α_1 zone. Most of the remaining activity was found in the α_2-zone. The amount of trypsin inhibited by the α_2-globulin fraction was 0.10±0.02 (S.D.) mg per ml serum.

Treatment of serum with 40 per cent acetone completely inactivated the α_1-inhibitor without simultaneous inactivation of the α_2-inhibitor and thereby allowed estimation of this inhibitor without electrophoretic separation of the trypsin inhibitors. The mean value of the α_2-inhibitor after acetone treatment was 0.13±0.02 (S.D.) mg trypsin inhibited per ml serum.

Sera from 38 individuals with α_1-antitrypsin deficiency were studied. Characteristic were the absence of the normal α band in paper or agar gel electrophoresis, a reduced mobility of α_1 antitrypsin on immunoelectrophoresis and reduction of total trypsin inhibitor capacity to 0.25±0.05 (S.D.) mg trypsin inhibited per ml serum. The mean trypsin inhibitor capacity due to α_1-antitrypsin in this deficiency state was 0.10 mg or 12 per cent of the normal level, while the corresponding capacity due to non-α_1-antitrypsins was the same as in normal sera.

Total serum trypsin inhibitor capacity was determined in sera from members of 14 families with one or more cases of α_1-antitrypsin deficiency. All together 213 individuals, including controls, were studied. α_1-antitrypsin deficiency was found to be a genetic defect transmitted by a recessive autosomal gene. The distribution of TIC among family members was found to be trimodal. In the homozygous state of α_1-antitrypsin deficiency the mean TIC was 0.24 and in the heterozygous state corresponding value was 0.67 mg trypsin inhibited per ml serum. In normal family members the mean was 1.10 compared with 1.07 mg/ml in 100 normals. The range of TIC in heterozygotes was defined as 0.40—0.80 mg trypsin inhibited per ml serum. There was no overlapping of the three groups of TIC values after exclusion of individuals with increased α_2-globulin fraction.

The frequency of the gene causing

without signs of pulmonary disease. Modifying genes may be responsible for the resistance to obstructive broncho pulmonary disease in such a family So far no evidence has been produced suggesting genetic heterogeneity of α_1 antitrypsin deficiency

The observations and considerations set forth above appear to warrant the tentative conclusion that hereditary α_1 antitrypsin deficiency predisposes to the development of obstructive broncho-pulmonary disease, especially emphysema, owing to an impaired resistance of the lung parenchyma to proteolytic enzymes possibly *liberated* in association with infections

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α_1 antitrypsin deficiency was calculated as 0 024 and the heterozygote frequency as 0 047

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No special type of chronic obstructive broncho-pulmonary disease was found to be associated with α_1 antitrypsin deficiency but patients with early onset of primary emphysema predominated α_1 antitrypsin deficiency appears to predispose to the development of chronic obstructive broncho-pulmonary disease, especially emphysema.

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FROM MEDICAL DEPARTMENT I, SAHLGRENSKA SJUKHUSET
UNIVERSITY OF GOTHENBURGH, SWEDEN

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PRIMARY RESULTS AND A FOLLOW UP INVESTIGATION

by

MAGNUS KORSGREN EILA LESKINEN VERONICA PETERHOFF ED BRADLEY**
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It is generally accepted that conversion of atrial fibrillation is of value, primarily to prevent atrial embolization and thrombosis, and to improve the hemodynamic effectiveness of the heart. (1 11 12, 35 37) Quinidine has been the drug of choice in treatment of atrial arrhythmias since its introduction. Consequently the established indications for conversion of atrial fibrillation are based upon the experiences with quinidine. The risk of deleterious ventricular arrhythmias due to the direct effect of large doses of quinidine on the myocardium at the moment of conversion has limited the use of this method of treatment (28 33 34). As a consequence, many patients may have been denied a conversion, when in fact conversion was indicated. The risk of embolization occurring at the time of conversion may have been previously overestimated. Untreated atrial arrhythmias are frequently accompanied by spontaneous embolization (7). In large series of conversions with quinidine, there has been no embolization reported which can be directly attributed to the conversion procedure (4 33)

An alternative to treatment with quinidine has been offered by the introduction of synchronized DC countershock (18 19 21) This method has rapidly gained widespread use (2, 6, 14 16, 21 22, 24 25 36).

The reports in the literature are in agreement concerning the effectiveness and safety of this method of treatment. A larger percentage of atrial arrhythmias can be converted to sinus rhythm with DC countershock than with the use of

quinidine. Information, however is lacking concerning the factors influencing the convertibility of different atrial arrhythmias in patients with different cardiac diseases. The prospect for the heart to maintain sinus rhythm after conversion also needs more elucidation. This is naturally due to a relatively limited number of patients treated and a short follow up time the world over. The present report is an attempt to analyze these factors in a series of 138 patients treated with DC countershock. *Analysis of the ECG from the short* pre- and post shock period as well as the acute hemodynamic alterations are *included*. *This series is a* continuation of a study on the effect of quinidine conversion in 350 patients with atrial fibrillation (5)

Method and procedure

We have used the Lown cardioverter (American Optical Company) in the manner recommended by LOWN *et al* (21) and also described earlier from this laboratory (36)

All the patients have been treated with adequate doses of dicumarol for at least three weeks prior to the first attempt of conversion. Manifestations of congestive heart failure have been treated in the conventional way to achieve the best possible steady state, with reference to heart rate, body water and electrolyte balance. All patients except 4 received digitalis prior to the shock. In an attempt to minimize the effect of electrolyte imbalance, the administration of diuretics was discontinued if possible, one week prior to the conversion attempt. Chlorothiazide diuretics were administered to 37 patients on at least one attempt of conversion early in the series.

The tolerance to quinidine has tested with a test dose of 0 1 gm quinidine sulfate per 10 kg of body w or with the administration of a - dose of a sustained release quinidine preparation (3 29) At the time of the conversion attempt no patient was premedicated with quinidine.

The patient was in the post absorptive state, having received a breakfast of bread and tea *approximately* five hours prior to the attempt of conversion. The conversions were performed in the car*dio-pulmonary laboratory with the pa*tient in a recumbent or semi recumbent position. A synthetic morphine prepara*tion (25 to 50* mg *of Petidin®) was used* as premedication administered subcutaneously one hour prior to the attempt of conversion. Methohexital (Brietal®) was used as an anesthetic in doses of 80 to 100 mg given intravenously within 20 seconds. Pure oxygen was given by effective manually assisted ventilation, even if no signs of spontaneously reduced respiration were noted. The shock was released after the eye lid reflex had disappeared. Additional 30 to 50 mg methohexital doses were injected if repeated shocks were required. After 6 to 11 minutes the patient was awake and could then be returned to the ward. Intubation and muscle relaxants were not *used in our material. In four cases pre*medication but no anesthesia was used. In these cases the amount of energy of the shock administered never exceeded 100 watt seconds (w.s.)

In addition to the cardioverter the patient was also connected to a three channel mingograph (Elema Schönander) A shock was released with the electrodes placed together forming a short circuit as a precautionary control of the

synchronizer. The position of the shock in the ECG was noted on the oscilloscope and a documentation on the writing mingograph (as a distinctly marked artefact) was then simultaneously obtained. After this control the methohexital was injected. The electrodes were firmly pressed against the thorax, one at the apex area and the other at the base of the heart. The last 23 patients in the series received the shock from a posterior paddle electrode placed on the inter scapular area. The frontal electrode on these occasions was placed somewhat to the right of the midsternal line at the level of the third to forth intercostal space.

The first shock was then released, the amount of energy generally varied between 70 to 170 w.s. If the first attempt was not successful, one or if necessary two additional shocks were administered with increasing energy up to 400 w.s. More than three shocks were not given in order to avoid burns of the skin. Recording of the ECG is impossible during the moment of shock without special arrangements for disconnection. However the ECG was recorded immediately after the release of every shock. Acceptable recordings were obtained within 3 to 5 seconds after the shock.

The pulsations of the arteries in the arms, legs and the carotid arteries were routinely checked before and after the procedure in order to discover a possible embolization immediately.

In the present analysis, a successful conversion is defined as the establishment of sinus rhythm, which was maintained for at least 24 hours. It was assumed that 24 hours were necessary to establish adequate maintenance treatment with quinidine. The establishment of sinus rhythm for a few beats or a few minutes is theoretically interesting but it has little practical value. Because of this criterion, our immediate results of treatment with DC countershock can not adequately be compared with the reports by other authors who considered a conversion attempt successful when at least one atrial contraction was normally conducted to the ventricle (2, 6, 16 19 24). Consequently 25 patients with sinus rhythm of short duration are considered as unsuccessful attempts of conversion in the present series.

In cases of successful conversions, maintenance therapy with quinidine was started within one hour after the shock. Quinidine sulfate 0.3 to 0.5 gm. was given as a first dose. Further maintenance therapy consisted of 1.0 gm. quinidine-bisulphate with sustained release (Duretter* Hässle, Göteborg, Sweden) twice a day (3 29). The patients who manifested intolerance to the test dose of quinidine, were given procainamide 2.0 gm. in four divided doses daily. Two patients successfully were converted but maintained sinus rhythm for only a few minutes. These two patients were later given a dose of 0.75 gm. quinidine-bisulphate with sustained release on the morning before the second attempt of conversion was to be performed.

Material

The material consists of 138 patients with atrial arrhythmias. 82 males and 56 females. The age varied between 30 and 73 years, mean age being 54 years. The majority of the patients had atrial fibrillation. Nineteen patients had atrial flutter and 4 patients had supraventricular tachycardia. One patient had a

slow atrial ectopic activity with a total AV block.

The distribution of diagnosis of the underlying heart disease in the series is shown in *table 1* The indications for an attempt to restore sinus rhythm with respect to underlying heart disease were liberal, in order to gain experience with the DC countershock method Patients with marked degree of mitral stenosis were not submitted to this treatment prior to valvutomy The group "combined mitral and other valvular lesions" consists of 10 patients with combined mitral valvular disease, 1 with aortic incompetence, 4 with combined aortic valvular lesions, 7 with combined mitral valvular disease and aortic lesions and finally 1 patient with mitral incompetence combined with aortic stenosis. The 46 patients referred to in the group of atherosclerotic heart disease were all more than 50 years of age. The majority of these patients had cardiac enlargement and/or ECG-changes indicating coronary heart disease. Some patients with arterial hypertension on antihypertensive therapy as well as patients with idiopathic atrial fibrillation are included in this group The group "others includes 2 patients successfully operated for a constrictive pericarditis, 1 patient

Table 1 Etiology of atrial arrhytmia and the primary results within different groups

Diagnosis	Total no. of patients	24 hours after DC countershock	
		no. in SR	per cent in SR
Mitral stenosis	16	15	94
Operated mitral stenosis	34	25	74
Mitral regurgitation	7	3	43
Combined mitral and other valvular lesions	23	19	83
Atherosclerotic heart disease	46	37	80
Other	12	8	67
Total	138	107	78
Previous quinidine failures	48	30	63
Previous quinidine failures excluded	90	77	86

with chronic alcoholism and 9 patients with cardiomyopathies of obscure origin.

The duration of atrial arrhythmia was recorded and is presented in connection with analysis of the convertibility in *figure 1*. In every case the heart volume was determined by the method described by JONSELL (15).

In this series 48 patients form a special group. These are patients obtained from the preceding study (5), who were submitted to a conversion attempt with quinidine but failed to establish sinus rhythm. The quinidine treatment was performed rigorously and it was discontinued because of failure to establish sinus rhythm or because of severe symptoms of intolerance to quinidine.

The treatment with DC countershock was performed on all 138 patients during the period from February 1963 to April 1964. The follow-up period covers the time from the establishment of sinus rhythm up to September 1964.

Results

Eighty five patients were submitted to treatment with DC countershock on only one occasion. Thirty six of these required more than one shock while the remaining 49 patients established sinus rhythm after the first shock was administered. Consequently 53 patients were exposed to two or more attempts of conversion. In the series as a whole, 230 attempts of conversion were performed. During these a total number of 440 shocks were administered.

In the total material 107 out of 138 patients were converted to sinus rhythm, that is a convertibility rate of 78 per cent. The best results were obtained in patients with mild mitral stenosis with a convertibility rate of 94 per cent. In the patients with surgically treated mitral stenosis the frequency of conversions was lower. Most of these patients had clinically and hemodynamically advanced cardiac disease. Only 3 of 7 patients with mitral incompetence established sinus rhythm following DC counter shock. This is in agreement with the results of treatment with quinidine published by others (12).

The relatively good result (80 per cent) noted in the group of patients with atherosclerotic heart disease agrees well with the results in a review of the literature by GOLDMAN (12) which includes a material of 1082 patients with atrial fibrillation treated with quinidine. The convertibility is, however, considerably lower among the patients with mitral valvular disease in GOLDMAN's review (50 to 55 per cent) as compared with the present results. The selection of patients has probably been based on different grounds and may therefore at least to some extent explain the differences between the results observed.

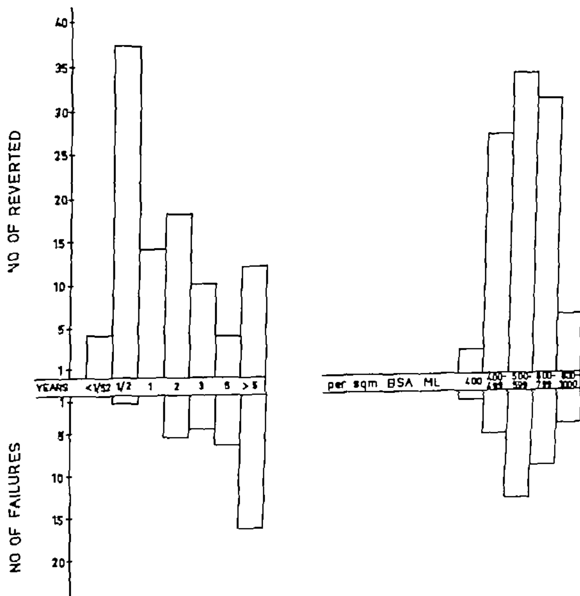
In the special group of 48 patients who previously failed to restore sinus rhythm during rigorous quinidine treatment, 30 patients have established sinus rhythm following DC countershock administration. Conversion rate in this group is thus 63 per cent. This relatively low figure might be dependent on the fact that these patients are generally more resistant to anti-arrhythmic treatment (both quinidine and DC counter shock) than the total unselected patient material. When this group of patients is excluded from the total material, the remaining patients will constitute such an unselected population of patients that might be seen in the future. The expect

ed convertibility would then be 86 per cent.

With respect to different types of atrial arrhythmias, it may be noted that 15 of 19 patients with atrial flutter and all patients with atrial tachycardia have established sinus rhythm with DC countershock. Conversion of the patient with an ectopic atrial activity with total AV block was successful.

The convertibility was analyzed with respect to the *duration of atrial arrhythmia*, *figure 1*. There is a trend that the *possibility of establishing sinus rhythm* with DC countershock is decreasing with increasing duration of the arrhythmia.

Figure 1. Results according to duration of preceding atrial arrhythmia and to roentgenological heart size



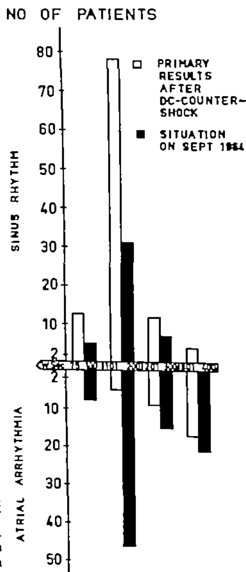
Only one patient with an atrial arrhythmia lasting less than two years was resistant to treatment. This was a 51 year old woman in function group III—IV with predominant aortic incompetence and a cardiac size of 700 mL/m.² BSA. On the other hand, 13 patients established sinus rhythm even though they had had the arrhythmia for more than five years. Decreasing convertibility rate with increasing duration of the atrial arrhythmia is well established from large series of arrhythmias converted with quinidine (12, 31 33).

The relation between convertibility and roentgenological heart size in ml./m.² BSA is also shown in *figure 1* No reversed correlation could be found between these two factors in contrast to the results obtained from quinidine series. Sokolow and Ball (30) demonstrated that increasing total heart size unfavourably influenced the convertibility of patients treated with quinidine. They could not find, however any correlation between convertibility and estimated atrial size.

Age and sex of the patients in this series did not influence the con ertibility Oram and Davies (25) reported a lower con ertibility in patients over 50 years of age.

The highest energy level used in dif ferent patients s related to convertibility n *figure 2* It is apparent that the majority of the patients were con erted to sinus rhythm by energy amounts of 200 w.s. or less. Only an additional 10 per cent of the successful con erted required energy levels higher than 200 w.s. It must be emphasized that 83 per cent of the patients were submitted to shock del ered by anteriorly placed electrodes and only the remaining minority with

Figure 2. Primary and follow-up results according to energy level at the conversion. For the successful ases the reverting setting is indicated For the unsuccessful attempts the highest energy level used is marked. (White staples demonstrate the primary result and black staples the situation at the end f follow-up investigation with an observation time from 4 1 19 months.)



anterior posterior paddles. Our experience with anterior posterior paddles is in agreement with the result reported by MORRIS *et al* (24) that these electrodes permit the use of lower energy settings to achieve successful results. The mentioned borderline of 200 w.s. will consequently be about 25 per cent lower using anterior posterior paddles which is in agreement with results reported by ORAM and DAVIES (25)

Follow up results and comments

The patients were usually discharged from the hospital on the 2nd or 3rd day following the conversion attempt. Relapse to arrhythmia caused the patient to return to the out patient department of the hospital. The time of relapse could thus be estimated accurately. The time of observation varies from 4 to 19 months.

The follow up investigation includes 100 of the 107 *successfully reverted* cases. Seven patients could not be reinvestigated. Four of these were lost to follow up while 3 had expired.

The results of follow up are summarized in *table 2*. Forty three out of 100 patients maintained the restored sinus rhythm at the end of the follow up investigation constituting a relapse rate of 57 per cent. A similar follow-up study by ORAM and DAVIES (25) covering the

Table 2. Results of follow-up investigation of the successfully reverted patients according to etiology of atrial arrhythmia.

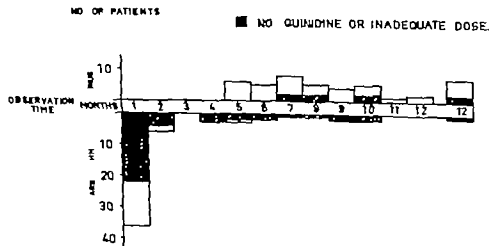
Diagnosis	No. of re-investigated primary successful cases	No. still in SR Sept. 1964	Per cent remaining in SR
Mitral stenosis	15	6	40
Operated mitral stenosis	24	7	29
Mitral regurgitation	2	1	(50)
Combined mitral and other valvular lesions	18	6	33
Atherosclerotic heart disease	33	17	52
Other	8	6	75
Total	100	43	43
Previous quinidine failures	26	8	31
Previous quinidine failures excluded	74	35	47

time between 1 to 11 months after DC countershock, shows a frequency of relapse of 52 per cent (44 relapses out of 84 converted arrhythmias). It is stressed that several patients have been followed for only one month. Comparison of these results with the present study at a follow up time between 1 and 15 months is of special interest, because only 32 of the total number of patients in the series of ORAM and DAVIES received maintenance therapy with quinidine. The remaining 68 per cent received digitalis as the only drug during the follow-up period. This comparison favours the present study which at that point of observation time disclosed 45 per cent of arrhythmia relapses. It is possible that the adequate quinidine maintenance during the follow-up period explains the better results in the present series.

The distribution of arrhythmia relapses with respect to diagnosis of the underlying cardiac disease is shown in *table 2*. Only 2 of 8 patients could not maintain sinus rhythm in the group "other". The patients who previously had failed to establish sinus rhythm during treatment with quinidine, influence the present result in the negative direction. Thus 69 per cent of these patients relapsed to arrhythmia. If this group of previous quinidine failures is excluded from the total material, the relapse rate in the remaining patients is 53 per cent.

The highest frequency of relapse seems to occur within the first two months after conversion, decreasing rapidly with time, *figure 3*. The majority of the 53 patients relapsing during the first two months were submitted to 2 or more attempts of conversion. Adjustments of the maintenance dose of digitalis, as well as quinidine were performed in these

Figure 3. Analysis of duration of sinus rhythm and the postconversion time for relapse to arrhythmia in the individual cases. The importance of an adequate quinidine therapy for maintaining sinus rhythm is shown by the few cases relapsing later than one month after conversion if they had adequate quinidine. (Filled portions of the staples illustrate inadequate maintenance-therapy of quinidine — see text.)



patients submitted for repeated conversion attempts. In some cases procainamide was added. These measures did not significantly improve the relapse rate in this group of patients. However four of these patients after several successful conversion attempts remain in sinus rhythm at the end of follow up study. This suggests that there is little to gain by repeated conversion attempts in patients who exhibit a tendency to relapse into arrhythmia within the first 2 months after conversion. This is also suggested by MORRIS *et al* (24).

A similar tendency of a high frequency of early relapses is also noted among patients with atrial fibrillation converted into sinus rhythm by quinidine (4).

The possible reasons for relapses were analyzed. The importance of maintenance treatment with quinidine is illustrated in *figure 3*. An adequate maintenance dose of quinidine is considered to be not less than 1.2 to 1.6 grams of quinidine sulphate per day which is based on the results from quinidine concentration studies in plasma (3, 4, 30). Thirty five of 43 patients remaining in sinus rhythm throughout the whole follow-up period had adequate maintenance with quinidine. Only 2 out of 15 patients who relapsed into arrhythmia later than 2 months after conversion had satisfactory quinidine medication. The reasons for inadequacies in quinidine administration were, either a failure in cooperation between the patient and the doctor or the appearance of intolerance to quinidine. Most common were untoward reactions from the gastrointestinal tract. This resulted in decreased doses or discontinuation of quinidine. Substitution of quinidine by procainamide did not prevent a relapse into atrial arrhythmia in the majority of these patients. These results suggest that adequacy of maintenance therapy with quinidine is of great importance in maintaining sinus rhythm established by DC countershock. This assumption is not valid if patients discontinuing quinidine medication for some unknown reason should constitute a selected group with more pronounced tendency to relapse. Consequently the present results are at variance with the results reported by ORAM and DAVIES (25) who questioned the necessity of this type of maintenance therapy. The follow up time of their series was shorter than in the present study and the number of patients observed was less.

Three out of 8 patients who retained sinus rhythm without quinidine therapy were taking procainamide, while the remaining 5 patients received neither quinidine nor procainamide. On the other hand, there were two patients who relapsed into arrhythmia despite adequate quinidine therapy. They displayed no obvious reasons for the relapse. Subsequently they were submitted to one more DC countershock which established sinus rhythm.

It can be seen in *figure 2* that none of the patients requiring an energy level of 300 w.s. or more maintained sinus rhythm to the end of the follow up investigation. This suggests again that administration of DC countershock of higher energy level is of questionable value, not only with respect to the immediate results, but also to the long term results. It must be mentioned that by using the now prevailing anterior posterior paddles, the useful energy level may be even lower. Thus the prognosis of maintaining the established sinus rhythm

can be estimated more or less accurately already during the first attempt of conversion.

Age and sex had no influence on the long term result. The duration of atrial arrhythmia preceding DC countershock which has been shown to influence the *immediate results proved* to be of major importance with respect to long term results as well. If all the primary successfully converted patients are grouped according to the duration of the preceding atrial arrhythmia in time intervals of up to 1 year 1 to 3 years, and over 3 years, the percentage remaining in sinus rhythm within these groups at the end of the follow-up period are 54 23 and 10 per cent respectively

The patients with a cardiac size of less than 500 mL/m.[2] BSA retained the established normal sinus rhythm in 40 per cent, while the corresponding number *in the patient group with a cardiac size* of 500 to 600 mL/m.[2] BSA was 28 per cent. No further increase in the rate of relapses was noted in patients with heart size greater than 600 mL/m.[2] BSA.

The degree of cardiac function, estimated according to the New York Heart Association, did not influence the patients ability to maintain sinus rhythm. The patients grouped as belonging to function group III and IV however are quite few in this series.

The situation in the primary patient material at the end of the follow-up pe

Table 3 Situation at end of follow-up period in primary selected patients

Diagnosis	Total no. of patients re-investigated	No. still in SR Sept. 1964	Per cent remaining in sinus rhythm
Mitral stenosis	16	6	38
Operated mitral stenosis	33	7	21
Mitral regurgitation	6	1	17
Combined mitral and other valvular lesions	22	6	27
Atherosclerotic heart disease	42	17	40
Other	12	6	50
Total	131	43	33
Previous quinidine failures	44	8	18
Previous quinidine failures excluded	87	35	40

patients submitted for repeated conversion attempts. In some cases procainamide was added. These measures did not significantly improve the relapse rate in this group of patients However four of these patients after several successful conversion attempts remain in sinus rhythm at the end of follow up study This suggests that there is little to gain by repeated conversion attempts in patients who exhibit a tendency to relapse into arrhythmia within the first 2 months after conversion This is also suggested by MORRIS *et al* (24)

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Three out of 8 patients who retained sinus rhythm without quinidine therapy were taking procainamide, while the remaining 5 patients received neither quinidine nor procainamide. On the other hand, there were two patients who relapsed into arrhythmia despite adequate quinidine therapy They displayed no obvious reasons for the relapse. Subsequently they were submitted to one more DC countershock which established sinus rhythm

It can be seen in *figure 2* that none of the patients requiring an energy level of 300 w.s. or more maintained sinus rhythm to the end of the follow up investigation. This suggests again that administration of DC countershock of higher energy level is of questionable value, not only with respect to the immediate results, but also to the long term results. It must be mentioned that by using the now prevailing anterior-posterior paddles, the useful energy level may be even lower Thus the prognosis of maintaining the established sinus rhythm

daily. She left the hospital 5 days after the last conversion, feeling much improved. Nineteen days after the conversion she suddenly without prior deterioration, expired in her home. Autopsy was not performed.

Case 2, E.D., 23 09 26

Diagnosis. Stenosis valv. mitrale et insuff. valv. aortae

This 40-year old man suffered at the age of 11 years from rheumatoid arthritis without signs of carditis. At the age of 18 years a systolic murmur at the apex region was heard but he remained asymptomatic. At 23 years he received a pension because of dyspnea and a relapse of acute rheumatic fever could not at this time be excluded. The following 14 years he experienced no change of symptoms and was in function group II. At the age 37 years the heart size was 640 ml/m. BSA. In the following year atrial fibrillation was discovered in connection with an acute cerebral embolism, resulting in a transient paresis of the right arm and a period of aphasia. An attempt of conversion with quinidine was performed. During the administration of quinidine he suddenly fainted. Because of this, the attempt was discontinued. During the following years he was continuously impaired. At the age of 38, his heart volume was 1050 ml/m. BSA, the enlargement above all localized to the left ventricle and left atrium. Calcifications were seen at the place of the aortic as well as the mitral valve. The physical findings agreed with a mitral stenosis combined with an aortic incompetence. This diagnosis was verified by heart catheterization and angiocardiography. He deteriorated the following two years to function group III. In 1964 at the age of 40 years he was given I[131] in a therapeutic dose to eliminate thyroid function. After that he improved very little. In February 1964 a conversion with a DC shock of 200 w sec. was successfully performed. He reverted to a regular sinus rhythm, but with pathological P waves indicating an intra-auricular disturbance of conduction (with a PR-interval of 0.26 seconds, decreasing to 0.24 second). He was treated with maintenance dose of 1.0 gm.×2 of quinidine, administered in the form of Kinidin Duretter®. In addition he was continued on Lanoxin 0.25 mg. and hydrochlorothiazide 0.25 mg. daily. There were no side effects of treatment and the patient left the hospital on the 7th day postconversion. After 4 day at home he suddenly died. Autopsy was not performed.

Case 3, M.O., 92 07 05

Diagnosis. Cardiosclerosis arteriosclerotica + status post infarctus myocardii + chronic heart-failure

The patient is a 71 year old man who enjoyed good health until 1 year before admission when he experienced chest pain of anginal type for the first time. During the last two months sensations of irregular cardiac activity appeared. He was admitted to the hospital acutely because of pulmonary edema which responded quickly to regular treatment. Physical examination of the heart did not reveal anything specific except tachycardia. The ECG disclosed a 2:1 blocked atrial flutter with a few ventricular premature beats. His condition improved after full digitalization and treatment with chlorothiazide. Atrial flutter persisted. One week later DC countershock of 200 w. s. was administered and atrial flutter with an atrial rate of 250 per minute and a ventricular rate of 126 per minute was reverted to sinus rhythm. The patient received 0.4 gm. of quinidine sulphate immediately after this reversion and additional 1.0 gm. quinidine bisulphate in sustained release tablets 4 to 5 hours later. Serum electrolytes were in balance. Some hours after DC counter shock, the patient developed complete AV-block with a ventricular rate of 66 per minute and an atrial rate of 65 per minute. During the following two days there was a regular ventricular rate of 57 to 63 per minute but no atrial activity could be detected on the ECG. Quinidine treatment with a dose of sustained release tablets 1.0 gm. in the morning and 1.0 gm. in the afternoon was maintained. On the third day after DC countershock regular sinus rhythm was manifested (PQ-time of 0.12 seconds and unchanged QRS-complex as compared with the preshock ECG which showed signs of posterior myocardial infarction). The following day the patient was discharged from the hospital with a maintenance dose of quinidine Duretter® 0.5 gm. twice daily and digitoxin 0.1 mg. per day. Ambulatory control 2 weeks later revealed normal sinus rhythm with a heart rate of 56 per minute, and PQ-time of 0.20 seconds. The patient was in good condition. Quinidine concentration in serum was 2.6 mg. per liter. No changes of the therapy were undertaken. Two days later the patient died suddenly without

riod is shown in *table 3* except for 7 patients lost to follow up It should be noted that the table includes the *successfully* converted as well as the unsuccessful attempts of conversion. Therefore, these results are closely related to the liberal criteria used in the selection of the primary patient material. One third of the primary patient material *remains in sinus rhythm* at the end of follow up A much smaller percentage (18 per cent) of the previous quinidine failures were remaining in sinus rhythm A higher percentage (40 per cent) remains at the end of follow up when the patients belonging to the previous quinidine failure group are excluded from the total series The group of patients with operated mitral stenosis deserves special comment, in that the group remaining in sinus rhythm at the end of the follow up period was lower than expected. This is in accordance with results recently reported (26)

No complications were noted which could be referred to the conversion attempt using the DC countershock. No peripheral embolism was observed during or immediately after the delivery of countershock even though several patients disclosed a history of earlier embolism It must be stressed that anticoagulant therapy of at least 3 weeks duration preceded all conversion attempts.

Six patients expired during the time of follow up study Three of them were resistant to DC *countershock while the* 3 others had been treated successfully and were maintained on quinidine. The deaths did not occur in close proximity to the conversion attempts. The histories of these patients are presented in the following chapter on case reports.

Case reports

Case 1 S.G

Diagnosis Status post restenosis valv. mitrale perata et status post embolia arteria iliaca bilat.

The patient is a 44-year old woman with erythema nodosum at age 6 years and acute rheumatic fever with carditis at 17 years of age. A completed pregnancy at 25 years of age was followed by transient symptoms of congestive heart failure. During the following years, symptoms typical for a pure mitral stenosis were increasing to such a degree, that indication for commissurotomy was present at age 31 She was much improved by the operation, but two years later a relapse of the rheumatic fever occurred. After the recurrence there was a rapid increase in heart size to 520 ml./m. BSA, with the predominant *enlargement being localized primarily* to left atrium and right ventricle. At the age of 37 years, atrial fibrillation and progressive symptoms of congestive heart failure developed. The following years an unsuccessful attempt of *conversion with quinidine was performed. Two* years later the patient was classified in function group III—IV having a heart volume of 770 ml./m. BSA. A second commissurotomy was performed after the diagnosis of restenosis of the mitral valve. She was improved and was now in function group I. A repeated conversion with quinidine was now successfully performed. Initially she had a first degree AV-block with PR intervals successively decreasing 0.60—0.28—0.24 seconds. On maintenance dose of quinidine she maintained this rhythm during the following three years, after which she relapsed into atrial *fibrillation. She again deteriorated to function* group III During continuous atrial fibrillation, she had an embolization episode requiring embolectomies from the right and left ilia arteries with good postoperative result. On half year later the first cardioversion was performed. (See *figure 7*) It was, however performed during a period of potassium depletion (3.0 meq./l.) induced by chlorothiazide. One week later a repeated attempt of conversion was performed after substitution of potassium to normal levels. The ECG recordings on these two occasions are demonstrated in *figure 7* After this last successful *reversion, she* was treated with a maintenance therapy of Kinidin Duretter® (1.0 gm.×2 of quinidine bisulphate) digitoxin 0.1 mg., Hygroton® 0.1 gm. and potassium chloride 3 gm.

daily. She left the hospital 5 days after the last conversion, feeling much improved. Nineteen days after the conversion she suddenly without prior deterioration, expired in her home. Autopsy was not performed.

Case 2. E.D. 23 09 26

Diagnosis: Stenosis valv. mitrale et insuff. valv. aortae

This 43-year old man suffered at the age of 11 years from rheumatoid arthritis without signs of carditis. At the age of 18 years systolic murmur at the apex region was heard but he remained asymptomatic. At 23 years he received pension because of dyspnea and relapse of acute rheumatic fever could not at this time be excluded. The following 14 years he experienced no change of symptoms and was in function group II. At the age 37 years the heart size was 640 ml/m. BSA. In the following year atrial fibrillation was discovered in connection with an acute cerebral embolus, resulting in transient paresis of the right arm and period of aphasia. An attempt of conversion with quinidine was performed. During the administration of quinidine he suddenly fainted. Because of this, the attempt was discontinued. During the following years he was continously impaired. At the age of 38 his heart volume was 1050 ml/m. BSA, the enlargement above all localized to the left ventricle and left atrium. Calcifications were seen the place of the aortic as well as the mitral valve. The physical findings agreed with mitral stenosis combined with an aortic incompetence. This diagnosis was verified by heart catheterization and angiocardiography. He deteriorated the following two years to function group III. In 1964 the age of 43 years he was given I[131] in therapeutic dose to eliminate thyroid function. After that he improved very little. In February 1964 conversion with DC shock of 200 w was successfully performed. He reverted to regular sinus rhythm, but with pathological P-waves indicating an intra-auricular disturbance of conduction (with PR-interval of 0.26 seconds, decreasing to 0.24 second). He was treated with maintenance dose of 1.0 gm.×2 *of quinidine*, administered in the form of Kinidin Duretter® In addition he was continued on Lanoxin® 0.25 mg. and hydrochlorothiazide 0.25 mg. daily. There were no side effects of treatment and the patient left the hospital on the 7th day postconversion. After 4 day home he suddenly died. Autopsy was not performed.

Case 3. M.O. 92 07 05

Diagnosis: Cardioarteriosclerosis + status post infarctus myocardii + chronic heart failure

The patient is 71 year old man who enjoyed good health until 1 year before admission when he experienced chest pain of anginal type for the first time. During the last two months sensations of irregular cardiac activity appeared. He was admitted to the hospital acutely because of pulmonary edema which responded quickly to regular treatment. Physical examination of the heart did not reveal anything specific except tachycardia. The ECG disclosed 2 1 blocked atrial flutter with few ventricular premature beats. His condition improved after full digitaliz tion and treatment with chlorothiazide. Atrial flutter persisted. One week later DC countershock of 200 w s. was administered and trial flutter with an atrial rate of 250 per minute and ventricular rate of 126 per minute was reverted to sinus rhythm. The patient received 0.4 gm. of quinidine sulphate immediately after this reversion and additional 1.0 gm. quinidine bisulphate in sustained release tablets 4 to 5 hours later. Serum electrolytes were in balance. Some hours after DC counter shock, the patient developed complete AV-block with ventricular rate of 66 per minute and an atrial rate of 65 per minute. During the following two days there was a regular ventricular rate of 57 to 63 per minute but no atrial activity could be detected on the ECG. Quinidine treatment with dose of sustained release tablets 1.0 gm. in the morning and 1.0 gm. in the afternoon was maintained. On the third day after DC countershock regular sinus rhythm was manifested (PQ-time of 0.12 seconds and unchanged QRS-complex as compared with the preshock ECG which showed signs of posterior myocardial infarction). The following day the patient was discharged from the hospital with maintenance dose of quinidine Duretter® 0.5 gm. twice daily and digitoxin 0.1 mg. per day. Ambulatory control 2 weeks later revealed normal sinus rhythm with heart rate of 56 per minute, and PQ-time of 0.20 seconds. The patient was in good condition. Quinidine concentration in serum was 2.6 mg per liter. No changes of the therapy were undertaken. Two days later the patient died suddenly without

any noticeable deterioration No autopsy was performed.

Case 4 C.K. 01 01 21

Diagnosis Atrial fibrillati n. Diabetes mellitus. Pulmonary emphysema.

This 63 year old dentist, from the age 39 years to 45 years, experienced several paroxysms of irregular cardiac activity of short duration. At age 46 years, persistent atrial fibrillation was converted to sinus rhythm with quinidine. Sinus rhythm remained only two days. However the patient was asymptomatic without therapy dur ing the following 15 years. Diabetes mellitus was detected at the age of 59 years and was easily controlled by chlorpropamide. Excessive alcohol consumption during the last 10 years was a problem. The atrial arrhythmia was treated with counter shock of 90, 300 and 400 w s. Sinus rhythm could not be established and the patient remained in atrial fibrillation with the same ventricular rate of 77 per minute. His heart size remained 410 ml./m. BSA. After the unsuccessful conversion he was fully digitalized and 3 days later discharged from the hospital with a maintenance dose of 0.2 mg digitoxin per day One week later the patient drowned during a fishing trip

Case 5. M. K. 18 08 02

Diagnosis Insuff et steno valv mitrale

This 45 years old woman was symptomfree until 42 years of age. There was no history of rheumatic disease but systolic pical murmur was known since 22 years of age. At age 42 years she started to complain of dyspnea on effort, palpitations and slight edema of the ankles. These symptoms began in connection with the appearance of trial fibrillation. She was digitalized and remained th following years in function group II. At age 44 years, increasing effort dyspnea led to a complete evaluation. The diagnosis of combined mitral valvular lesions with predominant incompetence was con firmed. She had a heart size of 800 ml./m. BSA with enlargement of the left atrium and left ventricle and pulmonary vascular congestion. The ECG showed left ventricular strain.

In December 1963 she was converted by shock of 100 w s. Maintenance with Kinidin Duretter® (1 gm.×2 of quinidine bisulphate) was started. Some hours after conversion, she had a transient period of Wenckebach s rhythm and in addition occasional supraventricular ex trasystoles. The following two days she remained in regular sinus rhythm with a heart rate of 65 to 70 beats per minute. (PR interval of 0.20 seconds.) Plasma quinidine levels were 2.1 to 2.8 mg per liter On the third postconversion night, she was found unconscious with no de tectable heart sounds. She improved quickly after a short period of external heart massage. Immediately after massage there was a bigemi nal rhythm which changed to regular sinus rhythm after a short period of time. Her plasma quinidine level was 2.5 mg. per liter and serum potassium level was 4 4 meq per liter The quinidine treatment was discontinued and substituted by procainamide 0.5 gm. in 4 daily doses. She remained on an unchanged dose of digitoxin 0 1 gm. day On this therapy she maintained sinus rhythm and left the hospital 14 days after the conversion. In February 1964 a relapse to atrial fibrillation was discovered and consequently the procainamide treatment was stopped. Her status remained unchanged during the following months. She expired at home, pobably after a short episode of acute pulmonary edema. The utopsy confirmed the diagnosis of mitral disease and a slight stenosis of the tricuspid valve. There was pronounced pulmonary edema but no evidence of coronary artery disease or embolization.

Case 6. P.S. 00 03 05

Diagnosis Atherosclerotic heart disease with congestive heart failure

This 63 years old man was previously healthy but had known atrial fibrillation since 1961 This appeared in connection with a pulmonary artery embolism after an operation for gastric ulcer The following three years he was rela tively free from symptoms on maintenance therapy of digitoxin. In 1963 increasing exer tional dyspnea developed and in October 1963 he was admitted to the hospital for an attempt of cardioversion. H had no signs of congestive heart failure during rest. Heart size was 550 ml./m. BSA and the ECG was normal except for trial fibrillation. The ECG during work agreed with coronary insufficiency with signi ficant ST T wave despressions. An attempt of conversion was performed Shocks of 120, 220 and 300 w s. were ineffective, induced no alteration in the rhythm. Further attempts were

considered useless and the patient left the hospital with the digitalis therapy unchanged. One month later the ECG was unchanged. His general condition was unchanged in comparison to that before the attempt of conversion, and he continued his ordinary work until he suddenly expired in his home in the end of April 1964. Autopsy was not performed.

These 6 patients histories constitute the 6 deaths in our total material of 138 patients. The first three related patients were successfully converted and were on maintenance therapy of quinidine. All of them suddenly died within a month after the conversion. Two of them had valvular disease in an advanced stage. The third was an older man, who had angina pectoris and a postinfarction ECG. The incidence of 3 cases of sudden death within a small group of 107 converted patients would seem to be significant. On the other hand, the spontaneous incidence of sudden death may be expected to be fairly high in a material of patients selected with respect to cardiac arrhythmias. Reports in the literature of similar materials of atrial fibrillation, reveal frequency of sudden death varying between 2.5 to 4 per cent (12). This relatively high incidence is also illustrated by the presence of 3 deaths in the material of unsuccessfully converted patients. Two of these patients died much later during the observation time, having no other maintenance therapy than digitalis. The third case in this group drowned a short time after the conversion.

Retrospectively no common connecting factor caused the mortality. The present incidence of deaths in this series must be considered to correspond to what might be expected during the relatively long time of observation in this material of patients with advanced heart disease.

Pre and postshock analysis of 75 electrocardiograms

A simultaneous depolarization of the whole heart is achieved by DC counter shock. This is followed by a short period of asystole. Sinus rhythm or different types of arrhythmias appear after this period or the pre-shock arrhythmia may persist. The duration of the arrhythmias showed wide variation, some of them ending as unchanged pre-shock arrhythmias, however the majority converting to sinus rhythm. The present analysis of the 75 electrocardiograms was aimed at describing the immediate post-shock rhythms after the depolarization when the heart starts its electrical activity. The frequency and types of these post-shocks arrhythmias have recently been described by LEMBERG *et al.* (17). Another reason for this investigation was to clarify if there was a correlation between these immediate post-shock arrhythmias to convertibility groups of diagnosis, ability to maintain the established sinus rhythm and energy level used.

ECG was recorded at 75 attempts of conversion in 62 patients both before and after DC countershock. Bipolar and unipolar extremity leads as well as unipolar chest-leads V1 to V6 were registered. In some additional cases intraesophageal and intracavitary electrodes were also used. Post-shock recording was started usually 3 to 5 seconds after the shock. Recording was continued until stable sinus rhythm was established. In patients with longstanding arrhythmias recordings were made periodically while the ECG was followed continuously on the oscilloscope.

Before the conversion attempts atrial

fibrillation was present on 62 occasions, atrial flutter on 12 occasions and finally 1 patient had paroxysmal supraventricular tachycardia. The conversion attempt resulted in sinus rhythm on 49 occasions of atrial fibrillation, on 10 occasions of atrial flutter and in the patient with paroxysmal tachycardia. The conversion attempts were thus unsuccessful on 15 occasions.

This electrocardiogram material was divided into 4 groups with respect to the post-counter shock rhythm. The *first group consists of 17 attempts which* resulted in a stable sinus rhythm immediately after the shock except for occasional atrial premature beats in a few cases, *figure 8*

The *second group consists* of 25 conversion attempts where sinus rhythm was primarily established but later was interrupted by periods of different types of arrhytmias. (*Figures 4 and 7*) The types of arrhythmias *seen in this group* were series of premature ventricular beats, episodes of supraventricular tachycardia, nodal rhythm sino-atrial or AV blocks of different degrees. Two or more of these rhythm *disturbances appeared on the* same ECG in some cases.

In the *third group* 18 conversion attempts are included where arrhythmias immediately *followed* the attempt and *were secondarily followed by the* establishment of sinus rhythm, *figure 6*

The *fourth group* consists of the 15 unsuccessful conversion attempts. On two occasions DC countershock did not influence the pre-shock arrhythmia at all. In the remaining 13 cases the same, previously mentioned, periods of arrhythmia were noted for a varying length of time after the shock (*figures 5 and 7*) On 7 of these 13 occasions short periods of sinus rhythm were noted but according to our definition these conversion attempts were considered as unsuccessful

Thus arrhythmias of different types and with different degrees of conduction disturbances occurred in 75 per cent of the material. On the other hand, no *serious post-shock complications* occurred, i.e. ventricular tachycardia, ventricular fibrillation or longer periods of asystole.

Further analysis disclosed that patients *with long duration of arrhythmia* (more than 5 years) had no greater incidence of postshock arrhythmias than the patients with arrhythmias of a shorter duration. This circumstance might be considered *surprising* One *patient with mi*tral incompetence and atrial fibrillation of 20 years duration established a stable sinus rhythm immediately after DC countershock of 200 w.s. On the other *hand another patient with operated* mitral stenosis and atrial fibrillation of 6 months duration restored sinus rhythm after a shock of 200 w.s. preceded by a period of total AV block and nodal *rhythm. This patient did not show* any clinical signs of digitalis intoxication or potassium depletion.

There was no correlation between the underlying *disease state and specific arrhythmias.* The distribution of cardiac diseases in the analysis of the ECG material was similar to that in the total patient material.

The incidence of relapses during an observation time of 1 to 7 months was rather high. Thirty two cases (53 per cent) in the ECG analysed material *relapsed during this short* interval of time. All relapses occurred within the first two months after conversion. Sev

enteen patients relapsed to the previous arrhythmias within the first week after the conversion. The distribution of the relapse incidence with respect to the above classification showed, that 8 relapses occurred in the *first group* consisting of 17 conversion attempts, 13 relapses were noted in the *second group* of 25 conversion attempts, and 10 relapses were recorded in the *third group* of 18 conversion attempts. Thus the same frequency of relapses occurred in all three groups. With respect to different types of arrhythmia, in 7 patients with bigeminal rhythm and 6 patients with nodal rhythm only one patient in each group was able to maintain sinus rhythm. This was the only significant pattern disclosed in the results of the ECG-analysis. This indicates that the tendency to ventricular arrhythmia and/or nodal rhythm has some relation to the duration of sinus rhythm established by DC countershock. Occurrence of bigeminal rhythm and nodal rhythm may be due to over-digitalization. However in these cases there was no objective evidence of over-digitalization.

The highest energy level required was 200 w.s. in the first group where stable sinus rhythm was established immediately after counter shock. In the second and third groups sinus rhythm was established by energy levels of 190 to 400 w.s. In the fourth group of patients where DC countershock did not change the preshock arrhythmia, the energy levels were 300 to 400 w.s. No other correlation between the amount of energy used and the different types of arrhythmias was observed. It may be noted, however that in the patients who were submitted to two conversion attempts, solitary premature atrial beats after the first conversion attempt at lower energy levels were replaced by supraventricular tachycardia on the second conversion attempt with increased energy levels.

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The *second group* consists of 25 conversion attempts where sinus rhythm was primarily established but later was interrupted by periods of different types of arrhythmias. (*Figures 4 and 7*) The types of arrhythmias seen in this group were series of premature ventricular beats, episodes of supraventricular tachycardia, nodal rhythm sino-atrial or AV blocks of different degrees Two or more of these rhythm disturbances appeared on the same ECG in some cases.

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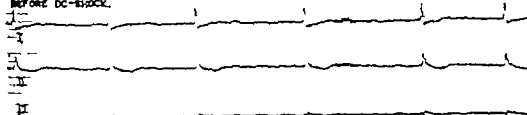
Thus arrhythmias of different types and with different degrees of conduction disturbances occurred in 75 per cent of the material. On the other hand, no serious post shock complications occurred i.e. ventricular tachycardia, ventricular fibrillation or longer periods of asystole.

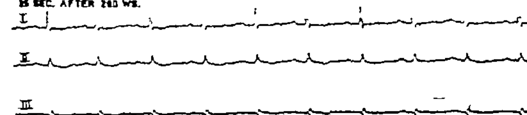
Further analysis disclosed that patients with long duration of arrhythmia (more than 5 years) had no greater incidence of postshock arrhythmias than the patients with arrhythmias of a shorter duration This circumstance might be considered surprising One patient with mitral incompetence and atrial fibrillation of 20 years duration established a stable sinus rhythm immediately after DC countershock of 200 w.s. On the other hand another patient with operated mitral stenosis and atrial fibrillation of 6 months duration restored sinus rhythm after a shock of 200 w.s. preceded by a period of total AV-block and nodal rhythm. This patient did not show any clinical signs of digitalis intoxication or potassium depletion.

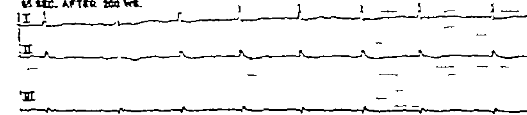
There was no correlation between the underlying disease state and specific arrhythmias. The distribution of cardiac diseases in the analysis of the ECG-material was similar to that in the total patient material.

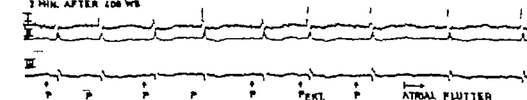
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fibrillation. A second conversion results in an ectopic atrial rhythm followed by ... rhythm before the ppearanc f atrial flutter







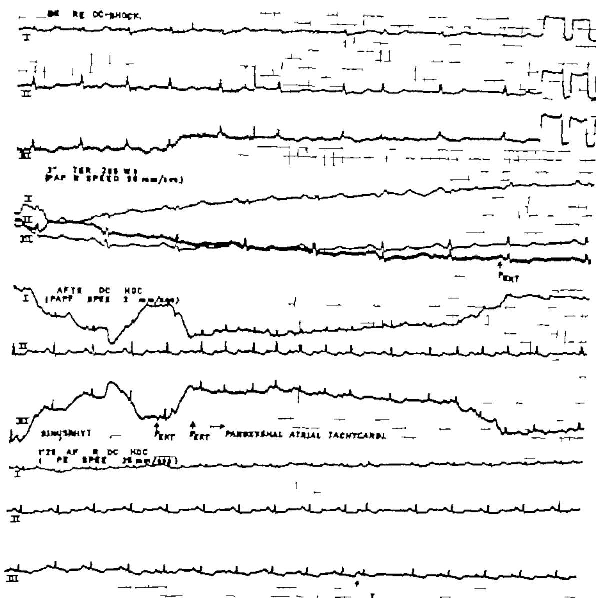


Case B.A. 11 04 16

This 47 year old female had mitral valvotomy in 1962. Atrial fibrillation had been present for 10 years. Her only therapy was digitalis.

On *Jan 1st, 1964* 3 DC shocks of 200—300—400 w were given. The 200 w shock resulted in short period of sinus rhythm, interrupted by an ectopic extrasystole originating from focus low down in the right atrium. The 300 w shock had no effect. After the last shock of 400 w atrial fibrillation remained. This was quickly followed by an ectopic atrial rhythm, interspersed with some beats from the sinus node. Two minutes post-shock, there wa spontaneous transition to sinus rhythm with sinus arrhythmia. After short period, an ectopic atrial extrasystole appears, which after one more sinus bea converts he nus rhythm to an atrial flutter

Figure 4 Conversion f atrial fibrillation to sinus rhythm, followed by a period of postconversion arrhythmia prior to finally establishing sinus rhythm.



Case N V 15 08 02

A 61 year old femal was dmitted with the diagnosis of rheumati heart disease, having had mitral valvotomy *in 1953 The duration of trial* f brillation was 3 months. She was treated with digitalis and chlorothiazide. On admission, Feb. 18th, and in the following 10 days, a slow irregular heart rate of 45 to 50 per minute was present in spite f discontinuation f digitalis on these days. With a work load of 500 kpm/min., the heart rate increased *to 95/min. Serum* potassium was 3.5 5.9 and 5.2 meq per liter

administered Immediately she had postshock regular sinus rhythm with only scattered trial *extrasystoles w th negative P w es in limb* lead I. One minute postshock period of rhythmia starts. At first, extrasystoles from the lower part of the right trium are seen and after that a paroxysmal period of tachycardia from the same focus. The middle of the period is in terrupted by a pause with two extrasystoles from nother ectopic trial focus. On and half min. postconversion there is a spontaneous

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BEFORE DC SHOCK
I
II

AFTER 220 WS
II
III

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BEFORE DC-SHOCK
I
II
III

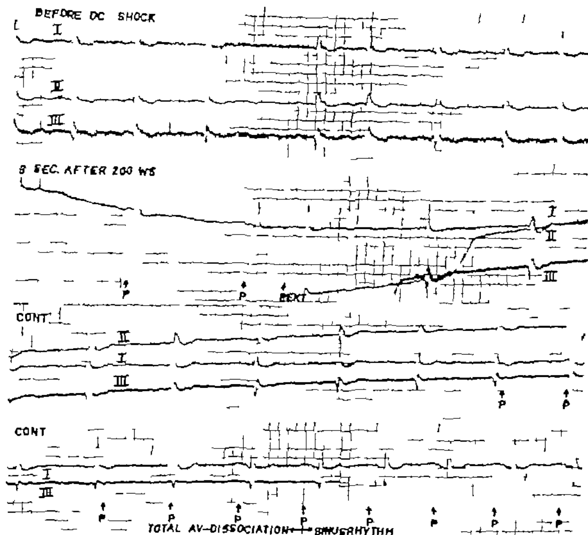
AFTER 200 WS
I
II
III
P P P EKT P EKT P P P P EKT P EKT P

NOV 30, 1963
I
II
III

Figure 7. Reversion of atrial fibrillation to an immediate sinus rhythm, followed by a postconversion period of ectopic atrial arrhythmia and disturbances of conduction prior to establishing sinus rhythm.

Fig e 6 Conversion of atrial fibrillation to sinus rhythm after a postconversion interval of nodal rhythm.

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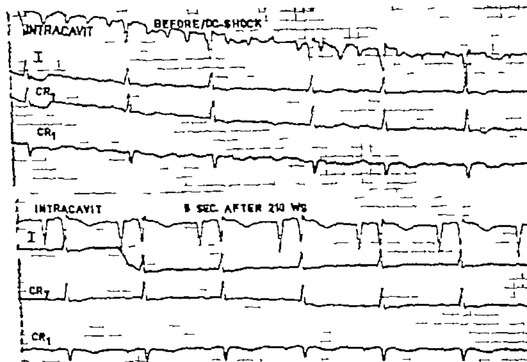


Case J.L. 00 12 20

This 64 year old female patient was admit ted with a diagnosis of mitral valvular disease, atherosclerotic heart disease and essential ar terial hypertension.

Atrial arrhythmia had been present for 4 months. She was treated with a maintenance dose of digitalis (Acylanide® 0.2 mgm. daily) Her ECG showed atrial fibrillation with a heart rate of 80 per minute DC shock of 200 w s. was gi en. After 3 seconds, nodal rhythm with a rate of 46 is recorded, quickly accelerat *ing to* a rate of 65 per minute. Immediately post-converiion, some P waves are visualized (complete AV-dissociation) but after an atrial *extrasystole, the P waves can not be seen, (with* reservation for retrograde P or sinus arrest?) At around 10 seconds, post-shock, P wa es reappear but AV-dissociation remains. Twenty seconds fter shock there is transition to sinus rhythm, coincident with the time, when the PR interval for the first time is 0.20 seconds Four hours late as well as during the following 3 days, she continued in sinus hythm with PR *interval of 0.28 seconds, but occasionally sinus* arrest with asystoles up to 3 seconds or with nodal escape beats were seen. From th fourth day and through the 8 months follow-up period, she has maintained regular si us rhythm on maintenance-therapy of only digitalis.

Figure 8 Conversion of atrial flutter to an immediate stable normal sinus rhythm.



Case H.R. 12 04 25

This 51 years old female had diagnoses of operated mitral stenosis and diabetes mellitus.

Atrial arrhythmia had been present for 6 years, treated with maintenance dose of digitalis.

The first attempt of conversion was performed in Sept. 1963 the patient then having atrial fibrillation with ventricular rate of 45 per minute. This attempt was unsuccessful. Three weeks later a second attempt with DC shock of 275 w. resulted in an atrial flutter which persisted on therapy with quinidine and digitalis. One week later DC shock of 120 w.s. reverted the flutter to sinus rhythm, but after 3 days, relapsed to atrial flutter with 4:1 block. A fourth attempt was performed one week later resulting in sinus rhythm. Four hours later atrial flutter reappeared after transient period of nodal rhythm with retrograde activation of the atria.

On Nov. 5th, the fifth attempt of conversion was performed (illustrated in the figure). Before shock, there was atrial flutter with alternating degrees of block and with ventricular rate of 48 per minute. Immediately after shock of 210 w.s., regular sinus rhythm appeared with PR-interval of 0.22 seconds and heart rate of 67/minute. She relapsed after only 7 days in sinus rhythm.

Two weeks later a sixth attempt of conversion was performed after having stopped digitalis and quinidine 7 days prior to the attempt. On this last occasion, she immediately reverted from atrial flutter to sinus rhythm which was maintained for 3 weeks. She then relapsed into atrial fibrillation and no further attempts were performed. A reason for the relapse after 3 weeks may be, that in spite of dose of quinidine bisulfate 1 gm. twice daily she had low level of plasma quinidine of 0.7 to 1.8 mg./liter

Case G.S 19 11 28

(The same as case 1 in the previous case reports.) This 45 years old patient had atrial fibrillation for 19 months. In the days before shock the patient continued in atrial fibrillation, sometimes with bigeminal rhythm. Heart rate at rest was 71/minute. With a work load of 400 kpm./min. there appeared several multifocal premature ventricular beats. Serum potassium was 3.0 meq./liter The treatment consisted of maintenance digitalis and chlorothiazid.

Nov 21st 3 DC shocks of 120—220—220 w s. After each shock, atrial fibrillation persisted interrupted by short periods of bigeminal rhythm and paroxysms of supraventricular tachycardia. Heart rate was around 120 per minute. Digitalis and chlorothiazid were discontinued for 7 days. The ECG showed no more ventricular premature beats and the level of serum potassium increased to 3.7 meq./liter

Nov 28th DC shock of 200 w s. Immediately after shock, a short period of sinus rhythm was recorded. After some seconds, multifocal atrial extrasystoles appeared of which some have negati e P-wa e in limb lead I (Focus in left atrium?) Then periods of atrial tachycardia are seen, with a frequency of 200 per minute, and with negative P in limb lead I. In addition, transient total AV-dissociation with nodal rhythm with a ventricular rate of 55 per min te and an atrial rate of 67 per minute is seen. During periods of total AV-dissociation, there ppears coupled, blocked, probably atrial extrasystoles with a negative P wa e in limb lead I, th first of these always appearing in the ST interval or in the T wave.

After these varying types of arrhythmia, the patient t last maintains sinus rhythm with first degree AV-block, with a PR-interval of 0.30 seconds.

case was given a long steady state analgesia with N_2O with muscle relaxants and controlled respiration.

Results

The results of this part of the investigation are recorded in *tables 4 and 5*. *Table 4* concerns the successfully reverted cases and *table 5* the cases resistant to conversion. The data from three of the four successfully converted patients investigated during catheterization are tabulated in three individual schematic drawings (*figures 11, 12 and 13*). On interpretation of the results, it is obvious that it is difficult to draw any definite conclusions because of the disturbing factors caused by the anesthesia. In addition to these factors, the patient's emotional state prior to the procedure may play a role. Thus the cardiac output either increased or decreased slightly after the conversion attempt in comparison to the values before anesthesia and conversion. A moderate decrease of the postconversion heart rate was generally noted which would imply an increase in stroke volume. On the other hand, a clearcut decrease of the cardiac output was noted in some cases as can be seen in *table 4*. This was especially apparent in those cases who had a large cardiac output before the procedure.

The systolic peak pressure in the brachial artery, as a rule, was slightly elevated (5 to 10 *mm Hg*) in the first

Table 4. Hemodynamic data of 11 successfully converted patients. + indicates successful shock, − an ineffective shock. Energy level is in watt-second (W). Change in flow (∠ CO) and stroke volume (∠ SV) is tabulated

Patient Age and diagnosis	Condition (supine position)	Pressures Brachial artery	Rh	Fr/ min.	CO l/min.	SV ml	∠ CO l	∠ SV ml	P_{CO}	pH
1	Rest	143/ 77 97	AF	86	6 7	78			34	7 48
K. E.	Anesthesia	144/ 76; 97	AF	63	—	—			—	—
54 yrs	Shock 75– 120 and 180 W +						−1.3	+17		
ASHD	Continued anesthesia	149 80 97	SR	60	—	—			31	7 44
	15 min post anesthesia, rest	136/ 75; 91	SR	57	5 4	95			43	7 46
2	Rest	135/ 79	AF	91	4.5	49			36	7 47
J. S.	Anesthesia	152/ 82;111	AF	73	—	—				
44 yrs	Shock 115 W +						+0.8	+ 9		
Oper MS+	Continued anesthesia	156 83 114	SR	97	—	—			39	7 44
AS+AI	15 min post anesthesia, rest	137/ 72 97	SR	91	5.3	58			42	7 42
3	Rest	153/ 80; 92	AF	67	6.2	93			35	7 44
P. R.	Anesthesia	178/ 98;124	AF	70	—	—				
57 yrs	Shock 100– 150 W +						−0 9	+17		
MS+MI	Continued anesthesia	137/ 76 99	SR	63	—	—			42	7 41
	15 min post anesthesia, rest	148/ 69 92	SR	48	5.3	110			36	7 46

Acute hemodynamic alterations during treatment with DC countershock

The introduction of conversion with DC shock has made it possible to study the acute changes during the very moment of conversion (13 23 27) Thus central and peripheral pressures throughout the conversion as well as cardiac output immediately before and after can be recorded This was previously impossible in conversions with quinidine, because the exact moment of conversion to sinus rhythm could not be predicted. Several publications (1 11 35) are available dealing with the hemodynamic alterations before and after conversion with quinidine. A slight increase in stroke volume and an increase in cardiac output at rest have been found. These signs of improved circulation became more pronounced on work, in the few cases studied (37) However these investigations do not deal with the acute changes and the effect of quinidine per se can not be isolated The investigations reported here were performed with medical therapy unchanged before and after the attempt of conversion. As a rule no more than 20 minutes elapsed between the two measurements of cardiac output. The direct effect of quinidine on the circulation can be excluded here. On the other hand, the anesthesia introduces a disturbing factor on the interpretation of the results.

Material Methods and Procedure

Cardiac output was measured in 18 patients before and after attempts of conversion The conversion was successful in 14 patients and failed in 4 cases. The arterial pressure was measured via a catheter introduced in the brachial artery by the percutaneous technique. The arterial pressure and heart rate were recorded regularly on at least five instances at rest in the supine position before anesthesia, during anesthesia, before the attempt of conversion during the conversion, in the immediate post conversion period and after recovering from the anesthesia Cardiac output measurements were performed using the dye dilution technique with intermittent sampling from the brachial artery catheter Bromsulphalein was usually used as the indicator The dye was injected through a catheter placed in the subclavian vein by the percutaneous technique. Four cases were investigated in connection with a diagnostic catheterization of the right and left heart. The pulmonary blood volume and mean transit time in the pulmonary circulation were determined by using the double indicator method described by FORSBERG (10) In some additional cases the dye was injected only through a catheter placed in the left atrium by the transseptal technique. In these cases the pressure was also recorded from the left atrium. In order to evaluate the influence of anesthesia, arterial pH and carbon dioxide tension were measured in several cases before and after conversion. In most of the cases cardiac output was measured immediately before and after anesthesia but in 5 patients, additional cardiac output determinations were performed during anesthesia before and after the attempt of conversions. The shortacting drug methohexital was used as anesthetic in most cases, but one case did not recieve anesthesia. Finally one additional

Table 5 Hemodynamic data of 4 patients during unsuccessful attempts of conversion.

Patient Age and diagnosis	Condition (supine position)	Pressures Brachial artery	Left atrium	Rh	Fr/min.	CO l/min.	SV ml	ΔCO l	ΔSV ml
1.	Rest	190/115 122	—	AF	60	3.6	60		
S.M-S.	Anaesthesia	186/122	—	AF	67	—	—		
52 yrs	Shock: 100- 150- 180 Ws-							—0.1	—24
MI+	Continued anaesthesia	178/122	—	AF	100	—	—		
Hyper	15 min. post anaesthesia, rest	187/125 146	—	AF	98	3.5	36		
2.	Rest	128/ 63; 88	—	AF	82	5.0	61		
O.M.	Anaesthesia	142/ 81	—	AF	72	—	—		
64 yrs	Shock 75- 120- 180 Ws-							—0.4	— 2
ASHD	Continued anaesthesia.	—	—	AF	82	—	—		
	15 min. post anaesthesia, rest	—	—	AF	78	4.6	59		
3	Rest	170 92 121	24	AF	82	4.1	50		
L.G.	Anaesthesia	123/ 72; 87	20	AF	72	4.8	67	—0.7	+17
59 yrs	Shock. 90- 150- 250 Ws-							±0	— 5
MS	Continued anaesthesia	169/86 114	22	AF	78	4.8	62		
4.	Rest	118/ 54 92	14	AF	45	3.2	71		
I.A.	Anaesthesia	120/ 59; 87	24	AF	41	2.2	54	—1.0	—17
51 yrs	Shock 100- 200- 400 Ws-							±0	+ 7
AS+	Continued anaesthesia	117/ 60 78	24	AF	41	2.5	61		
MI+MS									

Table 6 Hemodynamic data immediately before and after conversion from atrial fibrillation to regular sinus rhythm in 5 patients

	Rhythm	CO l/min.	Heart rate/min.	Stroke volume ml	$\bar{P}$ mmHg	$\bar{P}_{LA}$ mmHg
No of patients		5	5	5	3	5
	AF	4.3	114	44	29	18
	SR	4.4	85	52	22	14
Mean of change in per cent		+2	—23	+21	—20	—29
Range		+6 to —7	— 7 to —44	+61 to — 7	— 8 to —24	—10 to —53

Patient Age and diagnosis	Condition (supine position)	Pressures Brachial artery	Rh.	Fr/ min.	CO l/min.	SV ml	ΔCO l	ΔSV ml	P_{CO_2}	pH
4	Rest	172/108;136	AF	70	5.0	71			—	—
B. R.	Anesthesia	180/112	AF	66	—	—			—	—
54 yrs	Shock 100- 160 Ws +						—0.7	— 3		
ASHD+	Continued anesthesia	205/142 156	SR	67	—	—			—	—
Hyper	15 min. post anesthesia, rest	148/ 99;118	SR	63	4.3	68			—	—
5	Rest	156/ 80;104	AF	75	5.0	67			42	7 40
G G	Anesthesia	100/ 57	AF	104	—	—				
67 yrs	Shock 70 Ws +						—0.3	+ 3		
MS+MI	Continued anesthesia	149/ 71 94	SR	65	—	—			47	7.38
	15 min. post anesthesia, rest	160/ 80 108	SR	67	4.7	70			—	—
6.	Rest	156/ 78 98	AF	83	8 1	98			43	7 46
S. B.	Anesthesia	165/ 95	AF	112	—	—			—	—
33 yrs	Shock: 140 Ws +						—2.3	—15		
MS+MI	Continued anesthesia	168/ 89	SR	88	—	—			34	7 48
+AI	15 min. post anesthesia, rest	158/ 74 94	SR	70	5.8	83			—	—
7	Rest	166/ 79; 94	AF	40	3.8	95			—	—
K. G	Anesthesia	132/ 71	AF	72	—	—				
66 yrs	Shock: 50- 150 Ws +						+0.8	—16		
ASHD	Continued anesthesia	143/ 86	SR	74	—	—			—	—
	15 min. post anesthesia, rest	158/ 86;110	SR	58	4 6	79			—	—
8	Rest	129/ 77 90	AF	73	3 9	53			—	—
S I	Anesthesia, Shock 150 Ws +						±0	— 2		
43 yrs	Continued anesthesia	136/ 83 110	SR	80	—	—			—	—
Oper	15 min. post anesthesia, rest	124/ 79 92	SR	77	3 9	51			—	—
MS										
9	Rest	183/ 94 128	AF	75	—	—			—	—
R. M.	Anesthesia	190/114	AF	94	4.8	51			—	—
56 yrs	Shock 200 Ws +						+0.3	+10		
MI+	Continued anesthesia	194/114	SR	84	5 1	61			—	—
Hyper	15 min. post anesthesia, rest	163/ 92 107	SR	84	—	—			—	—
10.	Rest	128/ 65 88	AF	72	4.2	58			—	—
S. W	Anesthesia	93/ 53 71	AF	89	3 9	44	—0.3	—14	—	—
57 yrs	Shock 200 W +						+0.1	+ 4		
MS	Continued anesthesia	101/ 61	SR	83	4.0	48	—0.8	— 4	—	—
	15 min. post anesthesia, rest	110/ 66 84	SR	73	3.2	44			—	—
11	Rest	156/ 65 83	AF	40	2.9	73			—	—
A. V	Anesthesia	142/ 82 104	AF	100	2.8	28	—0 1	—45	—	—
64 yrs	Shock 200 Ws +						+1 7	+34		
Oper	Continued anesthesia	142/ 75 88	SR	73	4.5	62	—0.7	± 0	—	—
constr	15 min. post anesthesia, rest	145/ 75; 90	SR	61	3.8	62			—	—
pericard.										

cessfully converted patients, the pressure was recorded from the left atrium. The results in these patients are summarized in *table 6*. Three of the five are also illustrated in *figures 11, 12 and 13* and the two others are patients number 9 and 10 in *table 4*. The disturbing factors from the anesthesia in these patients are minimized as far as possible. One was given a steady state N_2O-analgesia with constant artificial ventilation, and two were investigated under continuous methohexital anesthesia. One received no anesthesia during the procedure. One of these patients had mitral regurgitation and arterial hypertension, while the other 4 were cases of mitral stenosis. One of the latter also had slight aortic incompetence. As can be seen in *table 6* there was no change in blood flow but a mean decrease of 23 per cent in heart rate. Stroke volume increased proportionately with a mean change of 21 per cent. In one of the patients, however the stroke volume decreased by 7 per cent. There was a prevailing decrease (mean change 20 per cent) of blood pressure in the pulmonary artery as well as in the left atrium where the mean decrease was 29 per cent.

There were no significant changes in the pulmonary blood volume in the three patients (*figures 11, 12 and 13*) where this estimation was performed before and after conversion and there was no prevailing tendency of change of mean transit time through the pulmonary circuit.

In summary conversion with DC shock gave a decrease in heart rate and a proportionate increase in stroke volume with unaltered total blood flow. There was also a decrease in the pressures in the pulmonary artery and left atrium and no significant change in the pulmonary blood volume.

2 to 8 beats. Except for this short interval it remained unchanged. The configuration of the arterial pressure curve in all cases immediately became normal (see *figure 9*) Even more pronounced alterations were noted in the pressure recordings from the pulmonary artery and left atrium in the 4 patients successfully treated during catheterization (3 are illustrated in *figures 11 12 and 13*) As can be seen in *figure 10* there was a momentary appearance of an a wave in the left atrial pressure curve and the appearance of a normal systolic wave in the pulmonary artery This may suggest a better performance of the right ventricle and a tendency to a more pulsatile flow in the pulmonary circuit. In 5 sne

Figure 9 Continous recording during cardioversion to regular sinus rhythm of pressure from a catheter placed in the brachial artery The moment of shock is indicated by an arrow It shows an increased peak systolic pressure an unchanged diastolic level and an immediate normalization of the configur tion f the pressure curve



F gure 10 C ntinous and simultaneous recording of p essures from the pulmonary artery and the left atrium. Both curves have the same amplitud t the ind ated standard of 50 mm f mercury a well as the same zero level. Befor shock the two curves follow each other cl sely Immediately after shock there s normalization of both curves with ppearanc of an -wave the left atrium reco ding

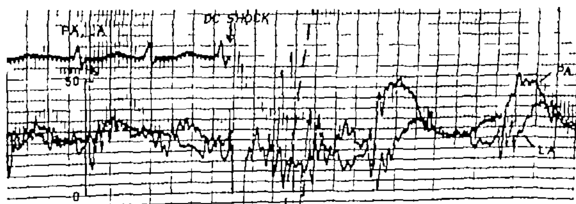


Figure 12. Blood pressures in brachial artery (BA) pulmonary artery (PA) and left atrium (LA), heart rate flow (CO), stroke volume (SV) pulmonary blood volume (PBV) and mean transit time (MTT) through the pulmonary circuit before and after successful reversion from atrial fibrillation to sinus rhythm in a patient with mitral stenosis. The moment of shock is indicated by an arrow on the time axis. The patient was in steady emotional state throughout the procedure and shock of 80 w s, was given without anesthesia

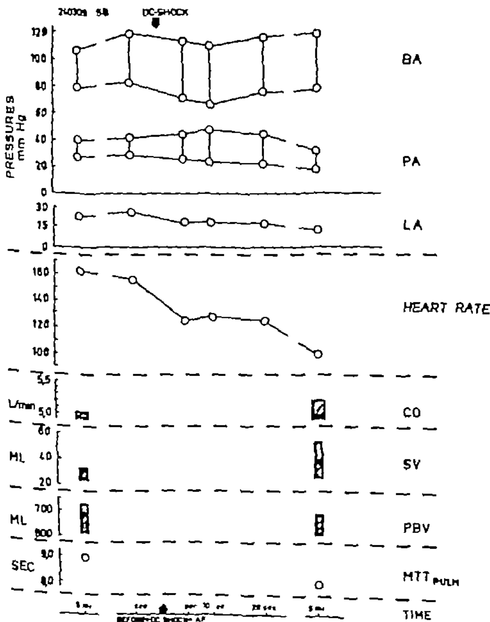
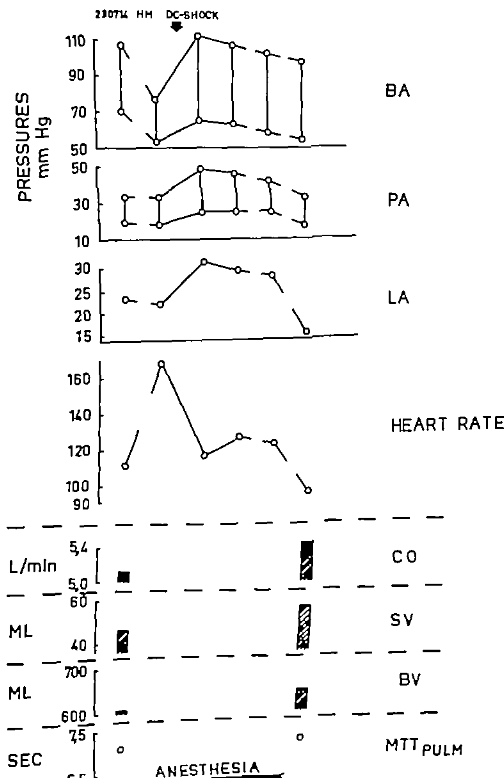


Figure 11 Blood pressures in brachial artery (BA) pulmonary artery (PA) and left atrium (LA), heart rate flow (CO) stroke volume (SV) pulmonary blood volume (PBV) and mean transit time (MTT) through the lungs bef re and after a successful conversion from atrial fibrillation to regular sinus rhythm in a patient with mitral stenosis and slight aortic insufficiency Time is indicated and the moment of shock is marked by an arrow The cardioversion was performed after an i. v injection f methohexital.

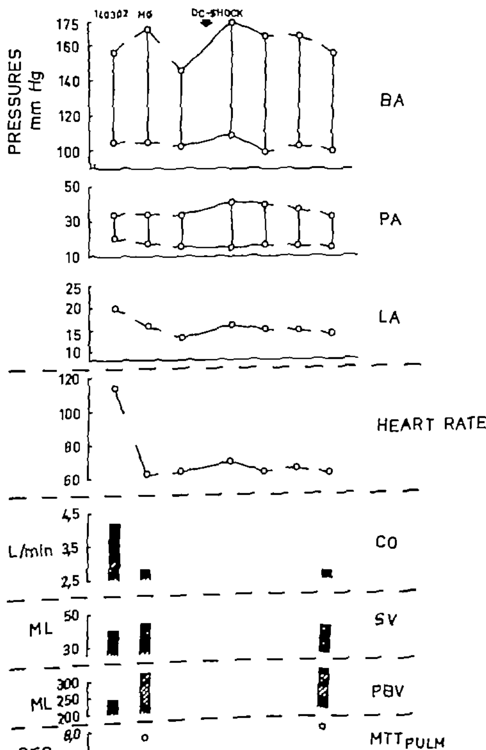


General conclusions

The present investigation demonstrated that DC countershock is a safe procedure in treatment of atrial arrhythmias if instructions are followed concerning the shock delivery in relation to the non-vulnerable phase in the cardiac cycle (the descending part of R or S-segment of the ECG). Precautions must be taken to minimize the artefacts on the ECG which may simulate the R wave and thus discharge the shock in the vulnerable phase of the cardiac cycle. The general anesthetic used in the study was methohexital which in some cases caused hiccup and in turn produced a marked artefact on the ECG. The discharge of the shock was therefore placed immediately after such an artefact in cases where it appeared. This precaution may explain the absence of any serious ventricular arrhythmia which has been described by others (16). It has been shown that the incidence of side effects occurring during methohexital anesthesia varied with the different types of premedication (8, 9). Petidin was used as premedication in this series because it significantly reduces the incidence of muscular twitching and hiccups.

The DC countershock procedure is time-saving with respect to the length of hospitalization of the patient as compared with quinidine treatment. It requires at least two doctors and one nurse during the performance and eliminates the requirement of continuous emergency set-up during several days of quinidine medication to cope with the rare but serious complications due to toxicity of quinidine on the myocardium (28, 34).

Figure 13 Blood pressures in brachial (BA) and pulmonary artery (PA) and left atrium (LA), heart rate flow (CO) stroke volume (SV) pulmonary blood volume (PBV) and pulmonary circuit mean transit time (MTT pulm) in a patient with slight mitral stenosis before and after conversion from atrial fibrillation to sinus rhythm. The point of DC shock is indicated by arrows on the time axis. N₂O-analgesia with muscle relaxants and by controlled ventilation was used in this case in order to have the patient in a steady state from the anesthetic point of view throughout the procedure. Thus the anesthesia started 15 minutes before the shock the patient was intubated and 10 minutes before and after the conversion all anesthetic steps were kept constant



with respect to diagnosis, convertibility heart size and age of the patient. The frequency of post chock arrhythmias, however were higher among the patients requiring higher energy levels of DC shock. It should be noted that the patients were not placed on anti-arrhythmic drugs prior to the delivery of the shock.

The patients were pretreated with dicumarol during a 3 weeks period prior to countershock in order to prevent possible embolization. There was no sign of embolism occurring in any of the patients after the conversion attempt.

The acute hemodynamic effects of DC countershock were studied in 14 patients establishing sinus rhythm and in 4 patients remaining in atrial fibrillation. The influence on basal conditions by general anesthesia and by the patient's knowledge, that he was to be submitted to DC countershock, limited the interpretation of these results. With respect to total blood flow the results were variable, probably dependent upon the different nonbasal conditions of the preconversion patient. However there was a decrease of flow in those patients who had a high pre-chock cardiac output. Including these patients, there was no mean change in total blood flow. There was a clear-cut decrease in heart rate and a proportionate increase in the stroke volume in the converted patients. In the group of unsuccessful attempts, there was no change in heart rate and stroke volume. The pressure curves recorded disclosed an increase in pulse amplitude suggesting an improved cardiac performance.

Despite the fact that the acute hemodynamic study did not show any dramatic hemodynamic improvement in patients converted into sinus rhythm with DC shock, it is reasonable to expect both hemodynamic and clinical improvement later on after the shock as it has been demonstrated by studies in patients converted with quinidine. The experience of relatively frequent embolization during different types of atrial arrhythmias probably constitutes the strongest indication to convert atrial arrhythmias into sinus rhythm. This can be easily achieved by DC countershock but the problem of keeping the patients in the established sinus rhythm remains, however practically unaltered as compared with the experiences after conversion with quinidine. Thus the most serious limitation for the use of DC countershock in every patient with atrial arrhythmia is our limited possibilities to maintain the established sinus rhythm. Quinidine, preferably in tablets of sustained release is still the best anti-arrhythmic drug. The present study as well as the earlier one stress the need of a more potent and more atoxic anti-arrhythmic drug. The patients in this material who were unable to take an adequate maintenance dose of quinidine showed a much more marked tendency towards relapse of arrhythmia.

The age span between 30 and 73 years of the present patient material suggests that DC countershock can be performed in all patients without respect to age. There seems to be no limit for shock application with respect to cardiac disease causing atrial arrhythmia. A wide range of roentgenological heart size was present in this material and there were no complications in patients with large hearts.

DC countershock was performed without general anesthesia in 4 patients. The problem of anesthesia has been discussed (20 32) Our experience is limited in this respect, but the general impression is that these patients were not as willing to submit themselves to a second shock attempt if needed as patients were who received general anesthesia. The procedure was characterized by them as unpleasant and in some instances painful.

The present results with respect to frequency of established sinus rhythm 24 hours after the shock are on the whole better than the results with quinidine treatment. The convertibility is higher with DC countershock than with quinidine in all groups of cardiac disease causing atrial arrhythmia. Patients with severe mitral stenosis were not submitted to a conversion attempt prior to surgical treatment of the stenosis. On the other hand this material includes a special group that had failed to establish sinus rhythm on quinidine treatment. This group had a lower rate of convertibility than the remaining patients in the total series This would indicate that DC countershock is superior to the quinidine method concerning the results at 24 hours after the administration of the shock. The convertibility was not influenced by the size of the heart or the age of the patient. The highest rate of conversion was found in patients with mild mitral stenosis but it was not much lower in patients with other cardiac diseases. The preceding duration of the atrial arrhythmia had a definite negative influence on the convertibility

The result of follow up in this series has shown a striking tendency for relapses to occur within the first 2 months after conversion. The prognosis for maintaining sinus rhythm seems to be good in any individual case, if the patient maintain sinus rhythm past this point of 2 months. In this series 43 per cent of the successfully converted patients remained in sinus rhythm at the end of follow up which is 33 per cent of the total patient series. A striking correlation (x^2 = 9 9 $p<0\,005$) was seen between maintaining sinus rhythm and the patients cooperation and/or his tolerance to quinidine therapy The long term results indicate that there is little value in using a greater energy level than 250 to 300 w.s in the primary conversion attempt. In this follow up investigation the duration of preceding atrial arrhythmia continued to influence the results, but other factors such as age, sex, heart size and function groups had less significant influence.

The result of the ECG-analysis on 75 conversion attempts disclosed that establishment of stable sinus rhythm could either follow immediately after DC countershock or be preceded by periods of varying types of arrhythmias. In some patients, arrhythmias which varied in type and duration interrupted an established sinus rhythm The postconversion ECG alterations did not differ within the different groups of patients

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Summary

One hundred and thirty-eight patients were treated in this series collected from a large general hospital in an attempt to ascertain the criteria to be used in the selection of patients for conversion with DC countershock The criteria used were liberal. The results were correlated to clinical data Alterations in the ECG in 75 consecutive attempts of conversion were analyzed and the acute hemody namic alterations were studied in 17 pa tients. The work includes a follow up study of from 4 to 19 months and con tains aspects on maintenance therapy with quinidine. There were no complica tions in connection with the conversion procedure.

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